ISTANBUL TECHNICAL UNIVERSITY ★ GRADUATE SCHOOL OF SCIENCE ENGINEERING AND TECHNOLOGY

FUNCTIONAL PROPERTIES OF PROTEIN ISOLATES AND ANTIOXIDANTS of TWO LOCAL TURKISH CRANBERRY BEAN VARIETIES AND *IN-VITRO* BIOACCESSABILITY STUDIES

M.Sc. THESIS

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İSTANBUL TEKNİK ÜNİVERSİTESİ ★ FEN BİLİMLERİ ENSTİTÜSÜ

TÜRKİYEYE ÖZGÜ İKİ YERLİ BARBUNYA ÇEŞİDİNE AİT PRTOEİN İZOLATLARI VE ANTİOKSİDANLARININ FONKSİYONEL ÖZELLİKLERİ VE İN-VİTRO BİYOULAŞILABİLİRLİK ÇALIŞMALARI

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Sayna ZAHEDINIA, a M.Sc. student of ITU Graduate School of Science Engineering and Technology student ID 506151538, successfully defended the thesis entitled "FUNCTIONAL PROPERTIES OF PROTEIN ISOLATES AND ANTIOXIDANTS of TWO LOCAL TURKISH CRANBERRY BEAN VARIETIES AND *IN-VITRO* BIOACCESSABILITY STUDIES", which she prepared after fulfilling the requirements specified in the associated legislations, before the jury whose signatures are below.

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To my family,

FOREWORD

First of all, I would like say thanks to my thesis advisor, Prof. Dr. Beraat ÖZÇELİK, for leading me toward my work, and improving my knowledge about food science. I also want to say my special thanks to Evren DEMİRCAN, Mine Gültekin Özgüen, Ümit ALTUNTAŞ, Eda ŞENSU who shared their knowledge and helped me during laboratory experiments. I also express my special thanks to my family.

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Sayna ZAHEDINIA
(Food Engineer)

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ABBREVIATIONS

PI : Protein isolate
ANOVA : Analysis of variance
DPPH : 1,1-Diphenyl-2- picrylhydrazyl
DW : Dry weight
HPLC : High Performance Liquid Chromatography
IN : After digestion dialyzed fraction of sample
IVPD : *in vitro* Protein Digestibility
OUT : After digestion non-dialyzed fraction of sample
SGF : Simulated Gastric Fluid electrolyte stock solution
SIF : Simulated Intestinal Fluid electrolyte stock solution
SSF : Simulated Salivary Fluid electrolyte stock solution
TAC : Total antioxidant capacity
TEAC : Trolox Equivalent Antioxidant Capacity
TFC : Total flavonoid content
TPC : Total phenolic content
CUPRAC : cupric reducing antioxidant capacity
FC : Folin-Ciocalteu
GAE : Gallic acid equivalent
GI : Gastro-intestinal
µg : Microgram
SD : Standard Deviation
TE : Trolox equivalent
Cb1 : Cranberry type 1(Oval)
Cb2 : Cranberry type 2(Kiraz)
Cb1PI : Cranberry protein isolate type 1 (oval)
Cb2PI : Cranberry protein isolate type 2 (kiraz)

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BIOACTIVE AND FUNCTIONAL PROPERTIES OF CRANBERRY FLOUR AND PROTEIN ISOLATE OBTAIND BY ISOELECTRIC PRECIPITATION METHOD

SUMMARY

Legumes play an important role in the human diet, especially in developing countries. They are a good source of protein and rich in dietary fiber, minerals, and vitamins. The different types of beans are a staple food and a cheap source of protein in many countries where protein malnourishment is widespread. The high cost and limited availability of animal proteins in that countries have increased interest to use legume proteins.

Legumes have been shown to have numerous beneficial physiological effects in the control and prevention of various metabolic diseases such as diabetes, coronary heart disease, and certain types of cancer. The beneficial influences of legumes may not be fully associated with dietary fiber, but phenolics and other non-nutritious compounds, polyphenols may act as antioxidants and hinder the formation of free radicals. Legumes belong to the food group that causes the least glycemic response. Thus legumes have earned the distinction as being good for health and this has led to the increase in their production. Thus legumes deserve the distinction of being good for health, therefore this has led to an increase in their production.

Although bean varieties are widely distributed around the world, data on the functional properties, bioactive compounds, and bioaccessibility of polyphenols are limited. The objective of this study is to study the functional properties of cranberry beans, analyze bioactive compounds value and, bioaccessibility, Giving the bioactive compounds profile of cranberry beans to increase interest in their use for food formulations.

Kidney beans Turkey is one of the most widely grown and consumed legumes. Usually grows in the Aegean region. It is an easy and easy to grow vegetable and one of the most grown products in the world. Dried and consumed as age. Kidney bean varieties are the most grown and consumed food in our country. Especially in winter, rice is the

crown of our tables with rice. Red bean varieties are easily grown in many regions because it is easily grown. It is suitable for dry and fresh use. If it is preferred to consume the freshness, it can be stored in canned or deep freezers during the winter season. available. In the kitchens, it is generally preferred to make red bean varieties with olive oil. It is food rich in vitamins. Red beans include iron, zinc, calcium, and vitamin E. It is beneficial for those with gastrointestinal problems because it contains high amount of fiber. Kidney bean varieties, which are such a useful food, can sometimes cause chest pain because they cause excessive gas and bloating. The aim of this study was to determine the functional properties and bioactive components of two types of cranberry beans grown in Nigde.

The protein content of the isolates was 86% by dry weight. Water holding capacity (WHC) of Cb1PI was 2.34 and Cb2PI 2.38 g of water/g of protein and OHC was 2.24 to 2.42 g oil/ g protein. In both types of beans, the stability of foaming was 100%.

Foaming capacity of Cb1PI was 40.4% and Cb2PI 41.2% and emulsifying capacity was 0.47% to 55.8%. In both types of beans, the stability of emulsifying capacity was 64%. The differences between functional properties of cranberry bean in literature may be is due to the difference between geographical differences like soil compounds the harvest and cultivation condition.

The variety results showed that the total phenolic content of cranberry flour ranged from 179.94 to 229.33 mg GAE/100g DW while the total phenolic content of their protein isolates ranged from 114.71 to 131.69 mg GAE/100g DW. Total phenolic content of flour was higher than the total phenolic content of the protein isolates, and this is for each of the varieties.

The total flavonoid content ranged from 171.88 to 198.58 CAE/100g DW for the flour of beans and 75.00 to 88.07 mg CAE/100g DW for the protein isolates. When compared TFC of flour and PI of cranberry beans, the PIs have lower TFC than the flour.

The total antioxidant capacity was measured by two different methods (DPPH and CUPRAC). When the results compare between two types of cranberry beans, type 2 (Kiraz) had the highest total antioxidant capacity. Antioxidant capacity of protein isolates in compare to flour of cranberry is low in both types of beans. In regards to the total antioxidant capacity methods, CUPRAC assay gave higher responses than the

DPPH assay. DPPH results for Cb1 were 191.09 and Cb2 were 114.14 TEAC/ 100g DW, CUPRAC results were 223.46 in Cb 1 and 165.6 TEAC/ 100g DW in Cb2.

The bio-accessibility of phenolics was assessed by *in vitro* gastro-intestinal digestion procedure that simulates the physiochemical and biochemical changes that occur in the gastrointestinal tract. Since in vivo studies on humans and animals are not preferred due to the limitations such as complexity, expensiveness and ethical reasons, *in vitro* digestion assays provide rapid and simple methods to evaluate phytochemical stability in food matrices. With the scope of this study, the bio accessibility of this local cranberry of Turkey is described for the first time.

Results from *in vitro* digestion demonstrated that differences in bio-accessibility of polyphenolics between two types of cranberry were significant. TPC increased in OUT fraction and, the recovery of TPC was quite high, 122% for Cb2 and 87% for Cb1, but the recovery of TAC by DPPH method was quite low, recovery of Cb1 antioxidant capacity by DPPH method was 2.3% and Cb2 was 1.2%. Additionally, recovery of TFC was 14% in Cb1 and 9% in Cb2. The recovery of TAC results of CUPRAC test was higher than DPPH, CUPRAC test results was 66% for Cb1 and 134% for Cb2.

Nine major phenolics were determined by HPLC system: Gallic Acid, ECGC, 4-hydroxybenzoic Acid, Syringic Acid, Caffeinne, Catechin, t-cinnamic Acid, Chlorogenic Acid, Ferulic Acid. They are shown in table 3.8 and table 3.9. the changes in peaks are so close in both type of beans.

ECGC ,4-hydroxybenzoic Acid, Syringic Acid, Caffeinne, was not detected in initial samples but detected after digestion in IN and OUT fractions and Gallic Acid and t-cinnamic Acid was in very low amount in initial sample but increased in IN and OUT fraction remarkably. ECGC is the major phenolic with 67.34 ppm level in IN fraction. The amount of phenolics increased after GI digestion, PH and enzymes effect may increase phenolic compounds during GI digestion. Chlorogenic Acid amount decreased during GI digestion.

Although the results obtained with the model of simulated *in vitro* GI digestion cannot directly predict the human *in vivo* conditions, still this model is helpful for investigating the bioaccessibility of phenolic compounds.

This study supports that, protein isolate extracted isoelectric precipitation from Cb which have possible application as functional ingredient during food manufacturing.

Especially, foaming and emulsifying activities and stabilities are remarkable functional properties comparing to OHC and WHC. WHC is functional property of proteins, which are important for soups, sauces, bakery products. OHC plays very important role like as mayonnaise or similar products due to emulsifying activity has close correlation with oil absorption capacity. CbPI could be promising emulsifying agent food, which needs high emulsification, and emulsion stability such as meat analogs, soups, salad dressings, mayonnaise, and cakes, plant based beverages. Experimental studies support that, CbPI might be great potential to find application in food products such as marshmallows, coffee whiteners, cakes, whipped toppings due to impressive foaming properties. Beside functional properties phenolic compounds, antioxidant capacity, and phenolic profile of beans are important. Nowadays, these properties of foods can attract people attention to plant protein consumption.

TÜRKİYEYE ÖZGÜ İKİ YERLİ BARBUNYA ÇEŞİDİNE AİT PROTEİN İZOLATLARI VE ANTİOKSİDANLARININ FONKSİYONEL ÖELLİKLERİ VE İN-VİTRO BİYOULAŞILABİLİRLİK ÇALIŞMALARI

ÖZET

Protein beslenme, gıda teknolojisi ve gıda işlemede önemli bir rol oynar. Hazırlık ve üretim sırasında depolama ve tüketimde; Proteinler, son ürünlere yapısal ve istenen özellikleri verir. Proteinler, gıda matrisindeki çözücüler, iyonlar, polisakaritler ve diğer proteinlerle etkileşime girer ve yiyeceklerin fiziksel yapısını değiştirir. Uluslararasında proteinlerin trendi çok fazla popülerlik kazanmıştır. Son beş yılda, dünya çapında üç kat protein talepleriyle yeni ürünler piyasaya sürülmüştür. Öte yandan, bitki protein tüketimi popülerlik kazanmaktadır, protein bakımından zengin ürünlerin tüketim oranı son on yılda hızla artmaktadır. Tüketiciler "doğal", "özgür" ve "sürdürülebilir" ürünler de dahil olmak üzere, giderek daha sorumlu, sağlık ve refah bir yaşam tarzı aramaktadır. Bitkiler doğada yüksek oranda bulunmaktadır, üretim maliyetleri ve işgücü hayvansal kaynaklara kıyasla düşüktür

Baklagiller insan beslenmesinde, özellikle gelişmekte olan ülkelerde önemli bir rol oynamaktadır. Bunlar iyi bir protein kaynağıdır ve diyet lifi, mineraller ve vitaminler bakımından zengindir. Farklı fasulye türleri, temel mal beslemesinin ve protein yetersizliğinin yaygın olduğu birçok ülkede ucuz bir protein kaynağıdır. Bu ülkelerde hayvansal proteinlerin yüksek maliyeti ve sınırlı bulunabilirliği baklagil proteinlerini kullanma ilgisini arttırmıştır.

Baklagillerin diyabet, koroner kalp hastalığı ve bazı kanser türleri gibi çeşitli metabolik hastalıkların kontrolünde ve önlenmesinde çok sayıda yararlı fizyolojik etkisinin olduğu göstermektedir. Baklagillerin faydalı etkileri diyet lifi ile tamamen ilişkili olmayabilir, ancak fenolikler ve diğer besleyici olmayan bileşikler, polifenoller antioksidanlar gibi davranabilir ve serbest radikallerin oluşumunu engelleyebilir. Baklagiller en az glisemik cevaba neden olan yiyecek grubuna aittir. Böylece baklagillerin sağlık açısından faydalıolduğu yönündeki görüşler üretimlerinin artmasına neden olmuştur.

Her ne kadar fasulye çeşitleri dünya çapında yaygın bir şekilde dağılmış olsa da, polifenollerin işlevsel özellikleri, biyoaktif bileşikleri ve biyoyararlanımı ile ilgili veriler sınırlıdır. Bu çalışmanın amacı, barbunya fasulyesinin fonksiyonel özelliklerini araştırmak, biyoaktif bileşiklerin değerini ve biyoyararlanımını analiz etmek, barbunya fasulyesinin biyoaktif bileşiklerinin profilini gıda formülasyonlarında kullanımlarına olan ilgiyi arttırmaktır.

Barbunya fasulye Türkiye de en çok yetiştirilen ve tüketilen baklagillerden biridir. Genelde ege bölgesinde yetişir. Yetişmesi çok kolay ve zahmetsiz bir sebze olup dünyada en çok yetiştirilen ürünlerden biridir. Kurutulmuş ve yaş olarak tüketilebilir. Barbunya çeşitleri, Ülkemizde en fazla yetişen ve tüketilen bir besindir. Özellikle de kış aylarında piriç pilavı ile birlikte sofralarımızın baş tacı olmaktadır. Barbunya çeşitleri oldukça kolay yetiştirildiği için bir çok bölgede kolaylıkla yetiştirilir. Kuru ve taze olarak kullanılmaya uygun bir sebzedir. Tazesinin tüketilmesi tercih edilirse kışın kullanmak amaçlı mevsiminde konserve yada derin dondurucularda saklanarak kış aylarında da kullanılabilir. kullanılabilir. Mutfaklarda genellikle zeytinyağlı barbunya çeşitleri yapımı tercih edilir. Vitamin bakımından oldukça zengin bir besindir. Barbunya çeşitleri içinde, demir, çinko, kalsiyum, ve E vitamini bulunur. Yüksek miktarda lif içerdiği için mide barsak problemleri olanlara faydalıdır. Bu kadar faydalı bir besin olan barbunya çeşitleri bazen de fazla miktarda gaz ve şişkinlik yarattığı için göğüs ağrısına neden olabilmektedir.

Bu çalışma Niğde'de yetiştirilen iki tür kızılcık fasulyesinin fonksiyonel özelliklerini ve çekirdeklerinin biyoaktif bileşenlerini belirlerlemek için amaçlanmıştır.

Izolatlarının protein içeriği kuru ağırlıkta% 86 olarak belirlenmiştir. Cb1PI'nin su tutma kapasitesi (WHC) 2.34 ve Cb2PI 2.38 g su / g protein ve OHC, 2.24 - 2.42 g yağ / g protein olarak bulunmuştur. Her iki fasulye türünde de köpürmenin kararlılığı% 100 olarak bulunmuştur.

Cb1PI'nin köpüklenme kapasitesi %40.4 ve Cb2PI %41.2 ve emülsifiye etme kapasitesi %0.47 ila %55.8 olarak bulunmuştur. Her iki fasulye türünde de emülsifiye etme kapasitesinin stabilitesi %64 olarak tespit edilmiştir. Barbunya fasulyesinin fonksiyonel özellikleri arasındaki literatürdeki farklılıklar, toprak bileşimleri gibi hasat ve ekim koşulları gibi coğrafi farklılıklar arasındaki farktan kaynaklanıyor olabilir.

Çeşitlilik gösteren sonuçlar, kızılcık unu toplam fenolik içeriğinin 179.94 ila 229.33 mg GAE / 100g DW arasında değişmektedir, protein izolatlarının toplam fenolik içeriğinin 114.71 ila 131.69 mg GAE / 100g DW arasında olduğunu göstermiştir. Unun toplam fenolik içeriği, protein izolatlarının toplam fenolik içeriğinden daha yüksek olduğu bulunmuştur.

Toplam flavonoid içeriği, fasulye unu için 171.88 ila 198.58 CAE / 100 g DW ve protein izolatları için 75.00 ila 88.07 mg CAE / 100 g DW arasındaydı. Unun TFC'si ve kızılcık fasulyesinin PI'ı karşılaştırıldığında, PI'lerin undan daha düşük TFC'si tespit edildi.

Toplam antioksidan kapasite iki farklı yöntemle (DPPH ve CUPRAC) ölçüldü. Sonuçlar iki tür barbunya fasulyesi arasında kıyaslandığında, Cb2 en yüksek toplam antioksidan kapasiteye sahipti. Her iki tür fasulyede de unu ile karşılaştırıldığında protein izolatlarının antioksidan kapasitesi düşük çıkmıştır. Toplam antioksidan kapasite yöntemleriyle ilgili olarak, CUPRAC testi DPPH testinden daha yüksek sonuçlar vermiştir. Cb1 için DPPH sonuçları 191.09 ve Cb2 114.14 TEAC / 100g DW, CUPRAC sonuçları Cb 1'de 223.46 ve Cb2'de 165.6 TEAC / 100g DW olarak tespit edilmiştir.

Fenoliklerin biyolojik olarak erişilebilirliği, gastrointestinal kanalda meydana gelen fizyokimyasal ve biyokimyasal değişiklikleri simüle eden *in vitro* gastro-intestinal sindirim prosedürüyle değerlendirildi. İnsan ve hayvanlar üzerinde yapılan *in vivo* çalışmalar, karmaşıklık, pahalılık ve etik nedenler gibi sınırlamalar nedeniyle tercih edilmediğinden, *in vitro* sindirim tahlilleri, gıda matrislerinde fitokimyasal stabiliteyi değerlendirmek için hızlı ve basit yöntemler sağlamaktadır. Bu çalışma kapsamında, Türkiye'nin bu yerel yaban mersininin biyoya erişilebilirliği ilk kez tarif edilmiştir.

İn vitro sindirimden elde edilen sonuçlar, polifenoliklerin iki kızılcık türü arasındaki biyo-erişilebilirlikteki farklılıklarının anlamlı olduğunu göstermiştir. OUT fraksiyonunda TPC artmış ve TPC'nin geri kazanımı oldukça yüksekti, Cb2 için% 122 ve Cb1 için% 87 bulundu, fakat DPPH yöntemiyle TAC'nin geri kazanımı oldukça düşüktü, DPPH yöntemi ile Tb'nin antioksidan kapasitesinin geri kazanımı %2.3 ve Cb2 % 1.2 olarak bulunmuştur. Ek olarak, TFC'nin geri kazanımı Cb1'de% 14 ve Cb2'de% 9 olarak bulunmuştur. CUPRAC testinin TAC sonuçlarının düzelmesi

DPPH'den yüksekti, CUPRAC test sonuçları Cb1 için% 66 ve Cb2 için% 134 olarak tespıt edilmiştir.

Dokuz majör fenolik HPLC sistemi ile belirlendi: Galya Asidi, ECGC, 4-hidroksibenzoik Asit, Şırınga Asidi, Kafein, Kateşin, t-sinamik Asit, Klorojenik Asit, Ferulik Asit. Tablo 3.8 ve Tablo 3.9'da gösterilmiştir. zirvelerdeki değişimler her iki fasulye türünde çok yakın bulunmuştur.

ECGC, 4-hidroksibenzoik asit, Syringic asit, kafein, ilk numunelerde tespit edilmedi, ancak IN ve OUT fraksiyonları ve GI'de sindirimden sonra tespit edilmiştir.

Simüle edilen *in vitro* GI sindirim modeli ile elde edilen sonuçlar doğrudan insandaki *in vivo* koşulları gibi tahmin edemese de, yine de bu model fenolik bileşiklerin biyolojik olarak erişilebilirliğini araştırmak için yardımcı olabileceği tahmin edilmiştir.

Bu çalışma, gıda üretimi sırasında fonksiyonel bileşen olarak kullanılması muhtemel olan protein izolatının Cb'den ekstrakte edilmiş izoelektrik çökeltisini desteklemektedir. Özellikle köpürme ve emülsifiye edici faaliyetler ve stabiliteler, OHC ve WHC'ye kıyasla dikkat çekici fonksiyonel özelliklerdir. WHC, çorbalar, soslar ve unlu mamuller için önemli olan proteinlerin fonksiyonel özelliğidir. OHC, emülsifiye edici aktivite nedeniyle yağ emme kapasitesi ile yakın korelasyona sahip olduğu için mayonez veya benzeri ürünler gibi çok önemli bir rol oynar. CbPI, yüksek emülsifikasyon gerektiren emülsifiye edici yiyecekleri ve et analogları, çorbalar, salata sosları, mayonez ve kekler, bitki bazlı içecekler gibi emülsiyon stabilitesi sağlayabilir. Deneysel çalışmalar, CbPI'nin etkileyici köpüklenme özellikleri nedeniyle krema, kahve beyazlatıcıları, kekler, çırpılmış krema gibi gıda ürünlerinde uygulama bulma potansiyeli olabileceğini desteklemektedir. Fonksiyonel özelliklerin yanı sıra fenolik bileşikler, antioksidan kapasite ve fasulyenin fenolik profili önemlidir. Günümüzde, gıdaların bu özellikleri bitki protein tüketimine dikkat çekmektedi

1. INTRODUCTION

In recent years, new doors to nutrition, and health have opened up with easy access to information on nutrition, plant engineering and medicine (Popescu and Golubev 2012).

Protein plays an important role in nutrition, food technology, and food processing. During preparation and production, storage, consumption; Proteins give structural and desired properties to end products. Proteins interact with solvents, ions, polysaccharides and other proteins in the food matrix and alter the physical structure of foods (Velazquez-Garcia et al. 2011).

Functional properties of proteins are important during consumption, processing, and storage, it is effective on the physical and chemical behavior of the food system (Schwenke 1996).

The trend of proteins internationally has gained too much popularity. In the last five years, new products have been launched around the world with triplicate protein claims. On the other hand, the plant protein segment is in bloom, with the consumption rate of protein-rich products has grown rapidly over the last ten years. Consumers are increasingly searching for a responsible, health and well-being lifestyle, including "natural", "free" and "sustainable" products. Plants are highly present in nature, their production cost and labor force are low in comparison to animal sources (Karaca, Frigerio, and Maechler 2011).

Terrestrial plants have been included in human nutrition to provide energy and nutrients for a sustainable lifestyle. However, vegetable proteins are less used than expected, although they are relatively cheaper and more abundant than animal proteins. In general, vegetable proteins are used in animal feed to recover milk, eggs, and meat. Considering obtaining animal protein (conversion to animal protein) is an inefficient method. The reason for this is that at most 15% of the protein feed of plant origin intended for human food in vegetable proteins, the rest being wasted at 85%.

Therefore, the production of animal protein for food is responsible for environmental pollution, and the production of animal protein, the production of animal protein requires 100 times more water than the same amount of water using for plant protein production.

The world's population is growing fast, and food security is one of the major concerns of the agricultural food industry. An effective conversation between plant sources and plant proteins can help find efficient and environmentally friendly energy supply solutions for human nutrition (Curran 2012; Hayat et al. 2014).

For instance, the development of new analog meat products, protein shakes and protein bars has been very popular recently. Some of the most promising alternatives are plant-based proteins, such as chickpeas, soybeans, peas and milk substitutes.

Plant protein-based meat and milk substitutes can provide the same quality but reduce costs by reducing greenhouse gas emissions and destroying woodlands (Buck, Day, and Trus 2013).

Pulses provide energy and potential health benefits, including reduced risk of cardiovascular disease, cancer, diabetes, high blood pressure, gastrointestinal disorders, and lowering of cholesterol LDL. Peas, chickpeas, beans and lentils have a protein content of 17-30% with a variable amount of essential amino acids. In recent years, it has been confirmed that proteins in a range of legumes are a valuable source of protein, probably as a type of food ingredient, because of their good functional and nutritional properties (Liu et al. 2015).

In human nutrition, beans highlighted great importance in counteracting malnutrition problems owing to their nutritive value, High contents of protein and easily digestible starch. In last decade, more attention has been devoted to potential health benefits of beans stemming from the presence of bioactive constituents. In addition to nutritional components, some common beans (*Phaseolus vulgaris* L.) are rich in several phytochemicals such as phenolic compounds especially including phenolic acids, flavonoids and tannins (Luthria and Pastor-Corrales 2006; Gulewicz et al. 2014),. Recently, Suárez-Martínez et al. (2016) reported the *Phaseolus vulgaris* L as a nutraceutical source for human health with favorable effects against cancer because of

the antimutagenic and antiproliferative properties of their phenolic compounds, lectins and protease inhibitors (Orak et al. 2016).

Given the nutritional value and profile of bioactive compounds in common bean, some countries have raised interest in their potential use in food formulations. In recent years, beans have gained increasing attention as a functional food or nutraceutical that can have many health benefits. Taking into account the nutritional and economic aspects of conventional beans, maize enrichment with bean flour has been studied to determine the technical feasibility of increasing nutritional value and it has been reported that extruded cornstarch could replace ordinary extruded snacks. a healthier option (Orak et al. 2016). In a similar study (Sathe 1996), the effect of adding common bean meal to semolina on the baking quality and total phenol content of pasta was evaluated (Orak et al. 2016).

The identification and quantification of phenolic compounds in beans is significant for the development of value-added bean products offering market opportunities in the functional foods and nutraceuticals sector. There is a great deal of interest in studying the phytochemical composition of legumes. Some researchers reported that phenolic compounds content and antioxidant activities of bean seeds were significantly affected by cultivar (Orak et al. 2016).

1.1 Purpose of Thesis

Although bean varieties are widely distributed around the world, data on the functional properties, bioactive compounds, bioaccessibility and profile of polyphenols are limited. There are many researches are available regarding to pulses and legume varieties, such as chickpea, pea, soybean, but limited or rare studies have been conducted regarding to *Phaseolus vulgaris* L, especially cranberry bean in the literature.

The objective of this study was to define the functional properties of cranberry beans and determine bioactive compounds profile of cranberry beans to increase interest in their use for food formulations and bioaccessibility of it.

1.2 Literature Review

1.2.1 Pulses and legumes

The term legume subsumes more than 12,000 different species, belong to the family Leguminosae. Among flowering plants, the Leguminosae is the third largest family (after Compositae and Orchidaceae) in size, and in economic importance second following the family of the grasses (*Gramineae*) (Sathe 1996). Legumes are a main food group in the diet of people around the world. Of the thousands of species, however, only a few are commercially grown: soybeans, dried beans, peas, cowpeas, beans, chickpeas and lentils. Soy is by far the most produced. There is no doubt that soy is the most valuable crop in the world. It is used by billions of farm animals as a source of protein food. Above all, oil is used by millions of people and in the industrial production of thousands of products. Because of this popularity, soy is usually separated from others (Sathe 1996; Sparvoli et al. 2016).Legumes are one of the most important categories of foods that have been widely used as staple foods in human history as a basic food aid for protein and energy needs. Recently, there is growing interest in public recognition of the health benefits of pulses for use in developed countries (Carbonaro, Maselli, and Nucara 2015; Chen, Bozzo, et al. 2015).

Albumin and globulin are the main proteins of legumes. Albumin is soluble in water. However, globulins are soluble in salts and account for about 70% of legumes (Siddiq et al. 2010). The legume is a hexamer protein with a mean molecular weight between 300 and 400 kDa (Garcia-Mora et al. 2015).

Common beans are rich in many phytochemicals that can have health benefits, such as polyphenols, fibers, lectins and trypsin inhibitors (Monk et al. 2016). Phenolic compounds are the main antioxidants contained in legumes. *In vitro* and animal studies have shown that phenolic compounds, in addition to the antioxidant effect, can also show other beneficial effects, such as. Anti-inflammatory, antihypertensive, anti-atherosclerotic and cytotoxic anti-tumor activities (Espinosa-Alonso et al. 2006; Hettiarachchy 2012). These vary considerably because of plant genetics, soil composition and growth conditions, maturity and post-harvest conditions (Wang et al. 2012).

In general, legumes contain high amounts of protein (Table 1.1). The highest protein contents are observed in lentil and cowpea varieties (Table 1.1). But lentils are more

popular both in terms of not only production but also consumption across the globe, and studies on lentils are more than other species.

Many varieties of common beans (*Phaseolus vulgaris* L.) are available with entirely different physico-chemical characteristics (Siddiq et al. 2010).

Table 1.1: Proximate composition of legumes mg per 100gr fresh weight

Species	Protein	Fat	ash	Carbohydrates
Cranberry	20.06-23.63	1.23-3.46	4.87	60-64
Cowpea	25-36	1-1.3	2-3.5	56-68
Pea	21.9-31	1.3-3	2-3.3	52-62
Chickpea	16-28	3.1-7	2-3.5	54-66
Lentil	20.6-32	3.1-7	2.5-3.4	54-58
Faba bean	24.3-32.3	1.1-4	2.2-2.8	57-60
Mung bean	23.3-27.7	0.7-2.4	3.2-4	61-62
Pigeon bean	15.9-24.1	1.2-1.6	4-4.5	57-58

It can be mentioned that legumes are not main source in terms of vitamin content (Table 1.2), but cranberry beans are rich source of folate.

Table 1.2: Vitamin content of different legumes (per 100gr fresh weight)(USDA)

Vitamin	unit	Species				
		Cranberry	Cowpea	Lentil	Pea	Chickpea
Vitamin C	mg	0	1.5	4.5	40	4.0
Thiamin	mg	0.74	0.85	0.87	0.27	0.48
Riboflavin	mg	0.21	0.23	0.21	0.13	0.21
Niacin	mg	1.45	2.08	2.23	2.09	1.98
Vitamin B-6	mg	0.30	0.36	0.54	0.17	0.54
Folate, DFE	µg	604	633	479	65	557
Vitamin A, RAE	µg	0	3	2	38	3
Vitamin A, IU	IU	2	50	39	765	67
Vitamin E (alpha-tocopherol	mg		0.39	0.49	0.13	0.82
Vitamin K (phylloquinone)	µg		5.0	5.0	24.08	9.0

1.2.2 Cranberry beans (Phaseolus Vulgaris L.)

Common bean (*Phaseolus vulgaris* L.) is the main type of legume with soybean for economic value and accounts for one third of global pulses output.(Garcia-Mora et al.

2015) common beans have gained attention for high phenolic content, decreased cardiometabolic risk elements, and a wide range of chronic diseases (Monk et al. 2016).

Common beans are main food in several Latin American and African countries. Mexico is the root of common beans; It was already consumed in the pre-birth period and the per head intake is 22 kg annually. After corn this type of legumes is the second essential source of carbohydrates, protein, minerals and vitamins. In Turkey approximately production of fresh beans 638.532 tones, production of cranberry bean 88.362 tons in 2017. The world fresh bean production was 21.720.588 tones while in Turkey it was 638.469 tons in 2014. On the other hand, in our culture beans are important for by replacing animal protein as an economical alternative and consumed indulgently by consumers. The cranberry bean is known for its creamy texture and flavor similar to chestnuts, and is a favorite in northern Italy and Spain. It has been reported that cranberry beans are low in fat and loaded with nutrients, and do not contain flatulence-producing enzymes (Aremu et al. 2015). They are also a magnificent source of nutraceutical components like protease inhibitors, fiber, phytic acid and polyphenols. The seed coat color is based on the amount of polyphenols namely condensed tannins, anthocyanins and flavonol glycosides. Their act is to preserve the seed from pathogens and predators (Espinosa-Alonso et al. 2006).

Figure 1.1 : Cranberry beans

1.2.3 Functional properties of food proteins

Proteins are a significant group of biological macromolecules found in all biological organisms consisting of elements as carbon, hydrogen, nitrogen, oxygen and sulfur.

All proteins are amino acid polymers. When classified based on their physical size, the proteins are nanoparticles Polymers, also named polypeptides, consist of a sequence of 20 different α-amino acids, also called residues. For chains of less than 40 residues, the term "peptide" is commonly used. For the purpose of complete their biological acts, proteins fold into one or more specific three dimensional shapes, arouse a series of noncovalent interactions like hydrogen bonding, ionic interactions, van der Waals forces, and hydrophobic packing (Hettiarachchy 2012). large number of the proteins consist of 20 types of amino acids. The combination of these amino acids outputs in the appearance of a substantial amount of proteins with various structures and functions. In principle, all proteins produced by organisms can be used by the human body. In the diet, though, only easily digestible, non-toxic and nutritious proteins with particular functions are used (Wang et al. 2012).

During the making and production, storage of proteins gives structural and desired properties. Proteins by interaction with solvents, ions, polysaccharides and other proteins in the food matrix and alter the physical structure of foods. Protein solubility, water and oil binding/absorption capacity, viscosity, foaming stability, emulsification, gelation are essential functional parameters, they are responsible for end product quality (Richter et al. 2016).

Functional properties of food proteins expressed as the physical and chemical properties, which are influence on protein behavior during production, storage, and consumption of food products. Protein structure, shape and surface hydrophobicity, hydrophobic / hydrophilic ratio, etc, aa acid composition, extraction and drying processes are several factors which mainly determinates functional properties. Solubility, water and oil absorption capacity, foaming and emulsifying, gelling are important functional features of food proteins and takes role constitution of food texture, organoleptic characteristics for confectionery, beverage, sauce and meat products (Boye, Zare, and Pletch 2010). The factors are influencing functional properties of proteins are indicated at Table 1.3

Table 1.3: Factors influence the functional properties of proteins in foods(Schwenke,1996).

Intrinsic	**Environmental Factors**	**Processing Treatment**
Composition of protein(s)	pH	Heating
Conformation of proteins	Oxidation-reduction status	Drying
Mono or multicomponent	Salts	pH
	Water	Ionic strength
	Carbohydrates	Reducing agents
	Lipids	Storage conditions
	Surfcatans	Chemical modification
	Flavours	

Thefunctional properties essential for the food producer, which provide desirable characteristics of the product at the suitable concentration of the respective components or additives and at proper conditions. These characteristics of proteins are exhibited in interactions with the surrounding solvent, ions, other proteins, lipids, and many other components, besides surface phenomena (Wang et al. 2012).

The sensory properties of foods arise out of the complicated interaction between various food elements. For instance, the flavor, texture, color and shape of a cake is provided by the combination of the foaming capacity, thermal gelation capacity, water holding capacity, emulsification, and browning reaction of the substances used. Hence, proteins as food materials must have multiple functional properties. Animal proteins, such as those derived from milk, egg and meat, are mixture of various protein components and therefore have different physical and chemical properties. For instance, egg white has good water holding, gelling, emulsification, foaming, and these features are provided by ovalbumin, ovomucin, conalbumin, lysozyme and their interactions. Plant proteins, like soybean and oilseeds protein, are combinations of various protein components (Wang et al. 2012).

It is notable that just a few types of beans seem to be appropriate for the development of specific food products, since, in addition to the texture, flavor is a critical characteristic that establishes the consumer approval of similar products (Sathe 2002).

1.2.4 Protein extraction methods

For study on structural and functional features of proteins, they must be isolated from the other proteins and non-protein compounds. The first step of protein extraction is separating of raw materials in proper solvent to gain protein rich product. alkaline extraction/isoelectric precipitation, water extraction, acid extraction, salt extraction and ultrafiltration, methods are frequently used to extracting pulse proteins. alkaline extraction by isoelectric precipitation is going to explained shortly in the following subsection (Boye, Zare, and Pletch 2010).

Aqueous alkaline extraction followed by isoelectric precipitation is another commonly used procedure for the extraction of legume proteins. The technique bases on the solubility of legume proteins, which is high at alkaline pH and low at pH values close to their isoelectric point (pH 4–5). Figure 1.2. shows schematic diagram of alkaline extraction and isoelectric precipitation process for production of pulse proteins extracts (Boye, Zare, and Pletch 2010).

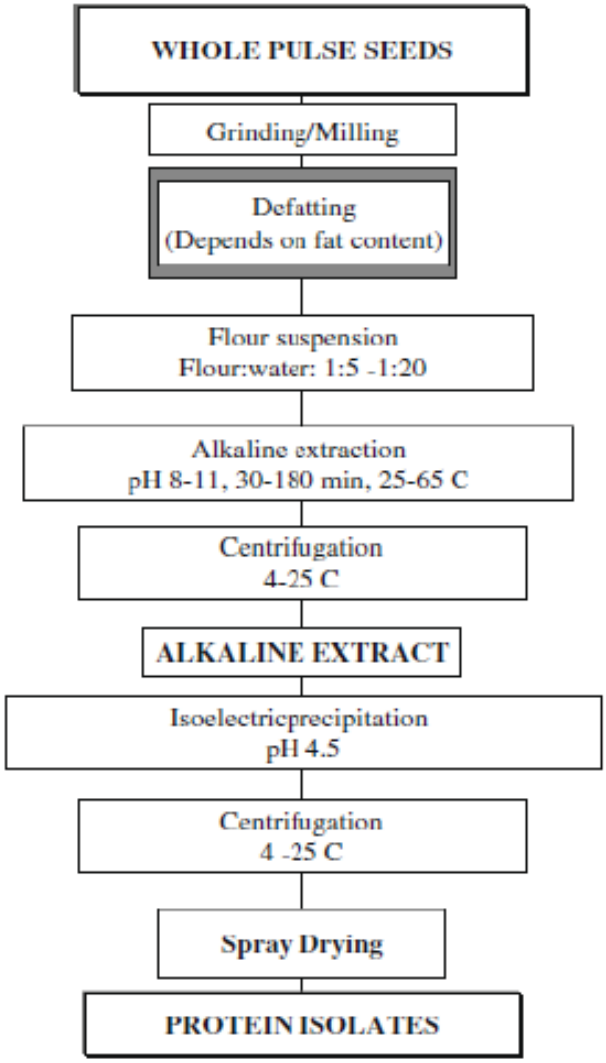


Figure 1.2: Schematic diagram of alkaline extraction and isoelectric precipitation process for production of pulse protein (Boye, Zare, and Pletch 2010).

1.2.5 Water and oil holding capacity

Protein can interact with water and oil because of its hydrophilicity and hydrophobicity in food matrix. Water-holding capacity (WHC) is a measure of the whole amount of water that can be absorbed by per gram of a protein isolate. This feature of protein is rely on the direct interaction of the molecules with water (Gyawali and Ibrahim 2016). The water holding capacity (WHC) can be defined as the ability to hold added water over the application of centrifugation, pressing, and forces (Zayas 1997). Water binding capacity is very important in food chain and new product development as well. If in the formulation there is not sufficient water is hold in food matrix serum separation occurs but in case of reverse condition, the sample get dry. The

determination of water holding capacity can be measure by suspending water the protein powder then emptying the water by centrifuging technique. Water holding capacity can be predicted with amino acid composition, which is related to proteins and very close relationship with amino acid profiles. Additionally, ionic charge residues, pH, temperature, ionic strength, and protein concentration change water-holding capacity (Fıratlıgil-Durmuş, Šárka, and Bubník 2018).

Dry bean proteins can be broadly classified as metabolic and storage proteins. The metabolic proteins include enzymatic as well as nonenzymatic proteins. Many of the dry bean storage proteins are often referred to as the globular proteins because of their globular shapes as well as the requirement of a certain amount of ionic strength (⌠) for their solubilization in aqueous media. However, certain globular proteins in beans are also partly soluble in water, perhaps due to ionic salt(s) associated with the protein(s) (Sathe 2002).

Oil binding capacity is effects quality parameters of foods. Hydrophobic amino acids in protein configuration can be promote for oil binding ability. Fat or oil binding capacity is calculated as the weight of oil absorbed per weight of protein powder or legume flour.

Fat/oil binding property of protein powder is also related to particle dimensions of the powders. Comparing to high having density protein powders with having low density and smaller particle size ones; low density and smaller particle size protein powders are absorbing, much more fat than higher dense protein powders. Most importantly, oil-binding mechanism gives information about protein microstructures (Fıratlıgil-Durmuş, Šárka, and Bubník 2018).

1.2.6 Emulsifying properties

Emulsion is two phase systems and prepared by solving or mixing two immiscible liquids as tiny (0.1 and 100 μm) droplets through the matrix of second liquid. It called emulsifying agent (Al-Malah, Azzam, and Abu-Lail 2000). The size of the droplets of the dispersed phase lies between. In addition, emulsion formation is correlated with surface activity and emulsifiers decrease interface tension and helps to enhancing stabile oil-water and air-water surface development (Fıratlıgil-Durmuş, Šárka, and Bubník 2018). Proteins show lower efficacy in lowering surface tension. However,

emulsions in which proteins are used as surfactants are more stable. Emulsifying property is important for many food applications such as soups, salad dressings, mayonnaise, and cakes. Emulsifying characteristic is highly influenced by the pH, ionic atmospheres, protein type, protein quantity, solubility, viscosity, variation in processing pre-treatment of the proteins and thermal processing of emulsion-based foods also external factors (Zhai et al. 2013). Among them, solubility is the one of the important feature of proteins among the other emulsifying features. Even the protein has less solubility, it forms weak emulsion stability (Ahmed et al. 2013).

1.2.7 Foaming properties

The property of proteins to make stable foams is significant for the making a wide range of foods. Foam are a two-phase system consisting of air cells divided by a thin layer of liquid, called a lamellar phase. Food foams are generally complicated systems, containing a mixture of gases, solids, liquids and surfactants. The distribution and particle size of the air bubbles is significant in appearance and structural features of foams, and it can affect them. Foams with even spearing of air bubbles give the body, smoothness and lightness. The proteins are responsible to even distribution of air in the foam structure. The body and the softness of food foams are related to the formation of air bubbles, which allows the evaporation of the aromas with a greater palatability of the food (Zayas 1997).

1.2.8 Phenolic compounds

1.2.8.1 Phenolic acids

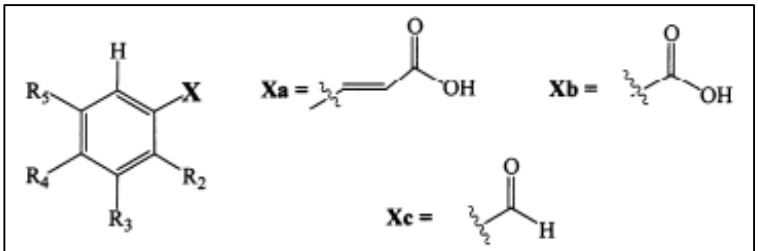
Phenolic acids separated into two types: benzoic acid and cinnamic acid (Robbins 2003). The naturally occurring phenolic acids structure are shown in Table 1.3. Most often type of hydroxybenzoic acids are, *p*-hydroxybenzoic, protocatechuic, gallic, and syringic acids having C6–C1 structure; while, hydroxycinnamic acids are aromatic compounds with a three-carbon side chain (C6–C3), with ferulic, *p*-coumaric and caffeic acids as the most significant representative compounds (Balasundram, Sundram, and Samman 2006b).

Legumes, have high amount of phenolic acids, especially which are dark colored like red bean, black bean, black eyed. The total phenolic content (TPC) of legumes are between 0.56-7.53 mg GAE/g FW; the greatest amount of total phenolic content was

indicated in seeds of black bean and lentil (Amarowicz and B. Pegg 2008; Sparvoli et al. 2016).

However, the quantity of these particular phenolic compounds can alter relying on the type of the legumes. The most frequently phenolic acid in legumes is p-Hydroxybenzoic acid and after that coumaric, procatechuic and ferulic acid (Magalhães et al. 2017; CHAVAN, AMAROWICZ, and SHAHIDI 1999). In pea, and chickpea the major phenolic acid is gallic acid (147-138 mg/100g FW, respectively) the major phenolic acid in lentil is coumaric acid (321-343 mg/100g DW) ben bean is trans-ferulic acid (341-367 mg/100g DW). protocatechuic acid (31 mg/100 g FW) in pea. Oboh (2006) reported that the total phenolic content of cowpea ranges from 0.3 mg GAE/g fw in a white cultivar to 1.0 mg GAE/g fw. in a brown variety, and likewise Gutierrez-Uribe *et al*., (2011) found a total phenolic content of 0.75 mg GAE/g dw. When compared with Kalogeropoulos *et al*. (2010), they found a lower total phenolic content for cowpea (0.15 mg GAE/ g fw) while Sreerama *et al*., (2012) reported higher amounts (12.16 mg GAE/g dw).

Gallic acid is the major phenolic acid in pea, and chickpea (148, 137 mg/100g fw, respectively) while coumaric acid is the major one in lentils (322-342 mg/100g dw) and trans-ferulic acid is dominant in bean (342-366 mg/100g dw). Those are followed by trans-p-coumaric acid (37.7-41.5mg/100g dw), protocatechuic acid (32 mg/100 g fw) in pea; p-hydroxybenzoic acid (93.6-100 mg/100g dw) and protocatechuic acid (49.9-52.3 mg/100g dw) in lentil; gallic acid (153 mg/100g fw), protocatechuic and p-hydroxybenzoic acid (32.8-41.4, 32.3-36.1 mg/100g dw, respectively) in bean; trans-ferulic acid (131 mg/100g fw) in chickpea. (Parmar et al. 2016)((Amarowicz et al. 2010)3: (Finn et al. 2016) (Amarowicz and Pegg 2008; Wojdyło, Oszmiański, and Czemerys 2007; Magalhães et al. 2017)



R_2	R_3	R_4	R_5	X	code	common name
H	H	H	H	a	1	cinnamic acid
–OH	H	H	H	a	2	*o*–coumaric acid
H	H	–OH	H	a	3	*p*–coumaric acid
H	–OH	H	H	a	4	*m*–coumaric acid
H	$–OCH_3$	–OH	H	a	5	ferulic acid
H	$–OCH_3$	–OH	$–OCH_3$	a	6	sinapic acid
H	–OH	–OH	H	a	7	caffeic acid
H	H	H	H	b	8	benzoic acid
–OH	H	H	H	b	9	salicylic acid
H	H	–OH	H	b	10	*p*–hydroxybenzoic acid
H	$–OCH_3$	–OH	H	b	11	vanillic acid
H	$–OCH_3$	–OH	$–OCH_3$	b	12	syringic acid
H	–OH	–OH	H	b	13	protocatechuic acid
–OH	H	H	–OH	b	14	gentisic acid
–OH	–OH	–OH	–OH	b	15	gallic acid
H	$–OCH_3$	$–OCH_3$	H	b	16	veratric acid
H	$–OCH_3$	–OH	$–OCH_3$	c	17	syringealdehyde
H	$–OCH_3$	–OH	H	c	18	vanillin

Table 1.4 : The structures of the major naturally occurring phenolic acids

1.2.8.2 Flavonoids

Flavonoids, are one of the leading classes in the phenolic compounds, occupy important antioxidant activity. They are the biggest group of plant phenolic compounds, and have over 50% of the 8000 naturally occurring phenolic compounds. Flavonoids consist of 15 carbon atoms, arranged in a C6–C3–C6 configuration so they have low molecular weight. flavonoids structure is composing of two aromatic rings A and B, joined by a 3-carbon bridge, mostly in the frame of a heterocyclic ring C (Figure 2.2). In the basis of replacement patterns to ring C, different results in the major flavonoid classes i.e. flavonols, , flavanones, , isoflavones, flavones flavanonols, and anthocyanidins (Figure 2.4) (Heim, Tagliaferro, and J. Bobilya 2002; Balasundram, Sundram, and Samman 2006b).

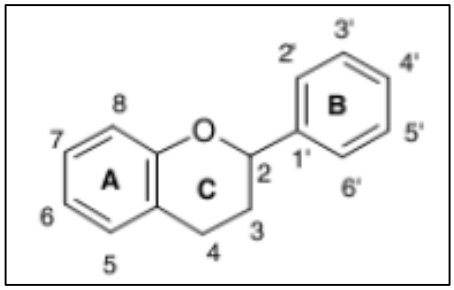


Figure 1.3: Structure of a flavonoid molecule(Balasundram, Sundram, and Samman 2006a)

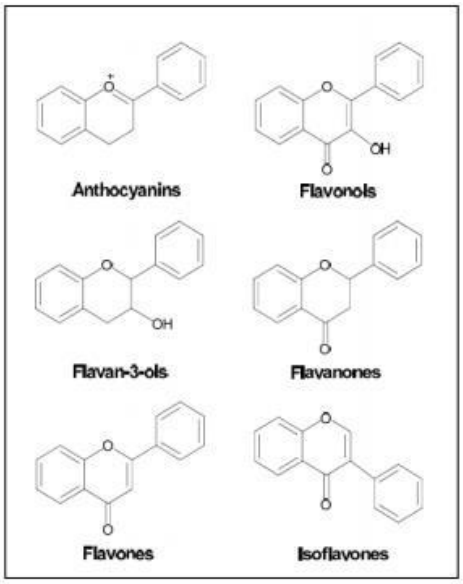


Figure 1.4: Major classes of flavonoids (Shahidi and Zhong 2015).

Flavonoids in foods are mainly as 3-*O*-glycosides and polymers. Flavonoids usually present in pulses belong to flavanols, anthocyanidin and flavones(Wang et al. 2011); quercetin, catechin, kaempferol, myricetin and luteolin and their derivate are frequently found. Quercetin 3-O-galactoside, kaempferol and catechin are forms, mostly present in pea; luteolin glucoside, quercetin glucoside and catechin in lentil; catechin, myricetin-3-O-rhamnoside and quercetin 3-O- in bean; myricetin-3-O-rhamnoside, quercetin 3-O-galactoside, quercetin-3-O-rhamnoside present in chickpea (Amarowicz et al. 2008; Amarowicz and Pegg 2008; Cai et al. 2003; Lopes et al. 2018; Castro-Guerrero et al. 2016).

The content of total flavonoids in seeds from legumes, cowpea, lentil, navy bean, black bean, and chickpea ranged from 0.09 to 4.54 mg CAE/g fw; the highest quantity of total flavonoids was determined in seeds of lentil and black bean (Ojwang et al. 2013).

found that red varieties of cowpea had the highest flavanol content (0.88- 1.06 mg CAE/g fw), whereas green and white varieties of it had the lowest (0.27-0.35 mg CAE/g fw). (De Ron et al. 2017)measured a total flavonoid content (TFC) of cowpea 0.27-2.09 mg CAE/g fw while (Santos-Zea, Gutiérrez-Uribe, and Serna-Saldivar 2011) reported that total flavonoid content of cowpea is 0.98 mg quercetin equivalents per g dry weight basis.

Flavonoids in foods exist primarily as 3-*O*-glycosides and polymers. Flavonoids generally present in legumes belong to flavanols, flavan-3-ols, flavones and anthocyanidin (Carbonaro, Maselli, and Nucara 2015); quercetin, catechin, kaempferol, myricetin and luteolin and their derivate are commonly found. Quercetin 3-O-galactoside,

kaempferol and catechin are forms, most dominantly present in pea; luteolin glucoside, quercetin glucoside and catechin in lentil; catechin, myricetin-3-O-rhamnoside and quercetin 3-O- in bean; myricetin-3-O-rhamnoside, quercetin 3-O-galactoside, quercetin-3-O-rhamnoside in chickpea (Carbonaro, Maselli, and Nucara 2015; Amarowicz and Pegg 2008; Castro-Guerrero et al. 2016; Magalhães et al. 2017; Parmar et al. 2016)

1.2.9 Antioxidant capacity

Antioxidants can terminate or defer oxidation and supply protection against some diseases associated with oxidative stress in the human body further various degenerative diseases, for instance after several studies on the significance of antioxidants in the body. biological systems by controlling oxidative stress causing diseases like atherosclerosis, diabetes mellitus, inflammation of chronic, neurodegenerative diseases (Karadag, Ozcelik, and Saner 2009a; Georgiev et al. 2010; Janiszewska-Turak 2014; Madrigal-Santillán et al. 2013; Vulić et al. 2012). Therefore, an excellent interest present in measuring and determination of antioxidant capacities of wide range of legumes.

Numerous methods have been developed and tried in several studies, but still strengths and weak points of these methods have not been find completely. No single assay precisely reflects the system of radical sources or all antioxidants (Shahidi and Zhong 2015). The antioxidant capacity methods divided into 2 classes; hydrogen atom

transfer reactıon (HAT) and electron transfer reactıon (ET) methods. Spectrophotometrically methods measuring a product formed by oxidatıon, associated with electron or radical scavenging, are most frequently used due to their plainness and fast reaction tımes (Karadag, Ozcelik, and Saner 2009a; Moon and Shibamoto 2009a) In the determination of the antioxidant activity of food components, DPPH assay is the most commonly and widely used method. DPPH readily forms a stable radical (DPPH), which accepts hydrogen from an antioxidant. The disappearance of DPPH, which is proportional to the antioxidant effect, is monitored by a spectrophotometer at 517 nm to determine antioxidant activity (Moon and Shibamoto 2009b).

1.3 Bioaccessibility

In human body, bioactive compounds obtained from the diet are extracted following a gastrointestinal (GI) digestion. The compounds must be biologically accessible to show the possible health benefits. Bioaccessibility specifically refers to the amount of antioxidants which are potentially presented to the intestinal brush border for absorption (Wootton-Beard, Moran, and Ryan 2011).In recent years, bioaccessibility studies have given evidence of the absorption of any phytochemicals and have shown that uptake varies widely, depending on the type of compound and/or food matrix (Manach et al. 2005).

In vivo studies on humans and animals are not preferred due to the limitations of complexity, expensiveness and ethical reasons. However, the *in vitro* digestion model gives an indication to the bioaccessibility since it is well correlated with the results obtained from human studies and animal models and a rapid and simple method to evaluate phytochemical stability in (Bouayed, Hoffmann, and Bohn 2011).The *in vitro* digestion model simulates stomach digestion using pepsin and mimickes intestinal digestion using pancreatin and bile salts.

2. MATERIALS AND METHODS

2.1 Materials

Two types of local cranberry beans called Oval and Kiraz (*Phaseolus vulgaris*.L.) were bought from local market, which were grown in Nigde province of Turkey.

Folin-Ciocalteu reagent, gallic acid, 2,2 diphenyl-1-picrylhydrazyl, Trolox (6-hydroxy-2,5,7,8-tetramethylchroman-2-carboxylic acid) were purchased from Sigma-Aldrich Chemical Co (Steinheim, Germany). Other chemicals and reagents were of analytical or high-performance liquid chromatography grade. For the simulation of *in vitro* gastrointestinal digestion system, dialysis bags (Dialysis tubing cellulose membrane, 1.3 in.) were purchased from Sigma-Aldrich Chemical Co (Steinheim, Germany). All chemicals and reagents used for the analyses were of analytical or high-performance liquid chromatography (HPLC) grade and obtained from Sigma-Aldrich Chemie GmbH & Co. KG (Steinheim, Germany), unless otherwise specified.

2.2 Methods

2.2.1 Determination of protein content in protein isolated of *Phaseolus vulgaris* L.

Protein content of dried flour specified regarding to AOAC methods. Total nitrogen measurement is carried out using Kjeldahl method. The method of (Amamcharla and Metzger 2010; Berner and Brown 1994)was employed considering modification associated with environment conditions. The chemicals were hydrochloric acid, sodium hydroxide, hexane, sulfuric acid, copper sulfate, potassium sulfate, boric acid, which were analytical grade. 1gr of sucrose was setted as blank. 0.2 gram of sample ,0.3 gram of copper sulfate, 15 gram of potassium sulfate were balanced into Kjeldahl tubes. These tubes containing were exposed to heating process for one hour under sulfuric acid (25 ml, 95%-98%) condition. Following the digestion step; test tubes were cooled to 25°C (room temperature) and 50 ml of distilled water was added to digested mixture. Then 25ml of boric acid with 2 drops of methyl red and 3 drops of methyl blue indicators were added. In the next Step, distillate was titrated using 0.2 N

hydrochloric acid solutions. Total protein nitrogen content could be computed with equation (2.1), 6.25 used as transıtion factor to switch nıtrogen to protein (2.2).

$$\text{Nitrogen}(\%) = \frac{0.014 nitrogen \times (mL\ HCL.sample - mL\ HCl,blank) \times normalityHCl}{g\ sample} \quad (2.1)$$

$$\text{Protein}(\%) = \%N \times 6.25 \quad (2.2)$$

2.2.2 Preparation of protein isolates (PIs)

Prior to protein extraction, samples were defatted by employing (Stone et al. 2015) method. In a brief summary, Cranberry flour was combined with hexane (1:3, w/v) for 40 min using a magnetic stir plate at 500 rpm tracked by decanting of the hexane. This method was then repeated for two times. After the last defatting, the combined extract was filtered through Whatman #1 filter paper then air dried for ~18 h in a fume hood. The defatted flour was stored at 4 °C.

Proteins were isolated according to the method of (Rui et al. 2011) with some modifications At the first stage, defatted flours were scattered in 15-fold Millipore water and shaken for 1 h at 55 °C, and pH adjusted to 9. Subsequently, the slurry was centrifuged at 11,000 rpm at 20 °C for half an hour, and the precipitates were discarded. The gathered supernatants were kept at 4 °C for 15 hours in order to obtain precipitated starch and then centrifuged using the method mentioned before. The result supernatant was exposed to acid precipitation by regulating the pH to 4.5 with the means of 1 M HCl solution. The sediments were washed with distilled water at 1:5 (w/w) for two times and centrifuges at 5000 rpm for 10 min then surplus of water omitted. Isolates were gathered and freeze dried and kept at 4℃ (Rui et al. 2011).

2.2.3 Determination of protein content of PI

Total content of nitrogen of freeze-dried isolated was specified by employing Kjeldahl method (Amamcharla and Metzger 2010; Berner and Brown 1994) was performed considering some modifications. Total protein nitrogen content figured with equation (2.1), 6.25 used as transition factor to converting nitrogen to protein, as equation (2.2)

2.2.4 Measurement of water absorption capacity and oil absorption capacity

Protein concentrate (0.5 gram) was relocated into a previously adjusted 15 ml centrifuge tube and 10 ml of distilled water was putted on it; The mixture was mixed at a rapid speed through a vortex mixer for 2 min in the next step. When the mixture was evenly wet and steady, it was chilled at the room temperature for half an hour, and then centrifuged at 5200rpm for 20 min. Next, the remaining supernatant was removed and the centrifuge tube consisting of sediment weight was measured. Water holding capacity was computed by the upcoming formula. where W0 is the weight of the dry sample (g), W1 the weight of the tube and the dry sample (g) andW2 weight of the tube plus the sediment (g) (Suresh Kumar et al. 2014)**.**

$$\text{WAC (g of water)} = \frac{W_2 - W_1}{W_0} \tag{2.3}$$

In favor of exploring the oil absorption capacities, 0.5 g of protein isolates was putted in a pre-weighed centrifuge tube and completely blended with 5 ml of sunflower oil. This protein–oil mixture was then centrifuged (5200rpm for 20 min); rapidly after centrifugation, the supernatant was conscientiously extracted and the tubes weight were assessed. OAC (grams of oil per gram of protein) was reckoned as if W0 is the weight of the dry sample (g), W1 is the weight of the tube plus the dry sample (g) and W2 is the weight of the tube plus the sediment (g) (Suresh Kumar et al. 2014).

$$\text{OAC (g of oil)} = \frac{W_2 - W_1}{W_0} \tag{2.4}$$

The process of oil absorption may be declared like a physical entrapment of oil associated with the non-polar side chains of proteins. Both the protein content and the type advanced the oil-retaining properties of food materials (Siddiq et al. 2010).

2.2.5 Measurement of emulsifying and foaming properties

In this part of experiments ,the applied method is a modified type of (DESHPANDE et al. 1983) . 0.1-gram sample putted into beaker then blended with 10ml water. pH tuned to 8 by employing 0.1 M NAOH. The mixture homogenized for 2 minutes, at room temperature hıgh speed blender (Ultra-Turrax T18 basıc, IKA, Staufen, Germany). Then 10 ml sunflower oil putted on it and homogenized process was performed for 2 minutes. Then slurry relocated to falcon tube and centrıfuged at

3300rpm for 5 mınutes at 20℃. To determine emulsifying stability, after the emulsıfication, samples were heated at 80℃ for half an hour, and then falcon tubes centrıfuged at 5000rpm for 5 mınutes.

Emulsıfying activity (EA) was figured as:

$$EA(\%)= \frac{Height\ of emulsified\ layer}{Height\ of\ contents\ of\ tube} \times 100 \quad (2.5)$$

$$ES(\%)= \frac{Height\ of\ \ remaining\ emulsified\ layer}{Height\ of\ initial\ emulsified\ layer} \times 100 \quad (2.6)$$

2.3 Phenolic Compounds Analysis

2.3.1 Extraction of polyphenols

Extraction of phytochemicals has been achieved by ultrasound-assisted extraction as follows: 0,5 g of lyophilized sample powder was combined with 5ml ethanol: water (80:20) and probe-sonicated for 20 s at 65% power using the ultrasound homogenizer Sonopuls UW 2200 equipped with the tip MS73 (Bandelin electronic, Berlin, Germany). During ultrasound-assisted extraction, samples were cooled in an ice bath to avoid sample heating. After centrifugation (6000 rpm, 5 min) the supernatant was separated and the solid residue was re-extracted twice with the same amount of extraction solvent. The extracts obtained were combined and made up to 15 mL with extraction solvent, and an aliquot was passed through a cellulose membrane filter.

2.3.2 Determination of total phenolic content (TPC)

Total phenolic content (TPC) were figures out through Folin-Ciocalteau assay. 100 µl of diluted samples extracted with 80% ethanol was combined along 0.2 N 1.5 ml of Folin-Cıocalteu reagent and 1.2 ml of 7.5% of sodium carbonate. The mixture was kept at room temperature in darkness for 90 minutes. The absorbance was measured at 765 nm through a microplate reader (Synergy HT, BioTek Instruments Inc., Winooski, VT, USA). A standard curve was obtained employing gallic acid and the samples were expressed on DW basıs as mg of gallıc acid equivalent (GAE) per 100 g of DW of sample. All samples were assessed in triplıcate (Spanos and Wrolstad 1990).

2.3.3 Determination of total flavonoid content (TFC)

Total flavonoid contents (TFC) were analyzed for cowpea flour extracts according to aluminum chloride method of Dewanto et al. (2002). In brief, 1 ml of extracts were mixed with 75 µl sodium nitrate (5%). After incubation for 6 min, 150 µl aluminum chloride (10%) added. After an incubation for 5min, 1M NaOH was added and left for 15min. The absorption of the solutions was measured at 510 nm using a spectrophotometer. Catechin was used as standard, and the total flavonoid content was expressed as milligrams of Catechin equivalents per gram dry weight sample (mg catechin/g dw).

2.4 Measurement of Antioxidant Activity

Antioxidant activıty of the samples was identified using 2, 2 – diphenyl-β-picrylhydrazyl (DPPH) free radical scavenging method and cupric decreasing antioxidant capacity (CUPRAC). Trolox was considered as standard for all antioxidant analysıs.

1.1.1 Measurement of antioxidant activity using DPPH assay

The process suggested by (Kumaran and Joel karunakaran 2006) was modified to 96 well microplate. First of all, 250 mL of DPPH solution was transferred to 15 mL of diluted sample solution and the mixture was kept at dark for 50 min. The absorbance was assessed at 515 nm by the end of time. Methanol was employed as blank and the result was gıven as % scavenging actıvity (SA %) and mM Trolox equivalent (Kumaran and Karunakaran 2006).

1.1.2 Measurement of antioxidant activity using CUPRAC assay

CUPRAC assay was carried out by using (Ribeiro et al. 2012) method. In the first place, 50 mL of 10 mM copper chloride solution was putted onto the well; then, 50 mL of 1 M ammonium acetate buffer at pH 7 was added and finaly 50 mL of 7.5 mM neocuproıne solution was added. After adding all reaction medium, 100 mL of diluted sample solution was added and the plate was kept at dark for half an hour. The absorbance was measured at 450 nm at the end of time. Distilled water was considered as blank and the conclusions were given as mM Trolox equivalent.

2.5 Simulation of *in vitro* Gastrointestinal Digestion

The *in vitro* measurement of bioaccessibility is regarded to be a reliable tool to approach the bioaccessibility of dietary compounds, taking into account eventual variations owing to food source, digestive stability of the compound, and maximum solubility in the gastrointestinal medium, as an index of availability for eventual processes of apical uptake by absorptive epithelial cells (Viganò et al. 2008).

The *in vitro* gastrointestinal digestion model was a slightly modified version of the technique developed by (Bermúdez-Soto, Tomás-Barberán, and García-Conesa 2007; Lo Curto et al. 2011)was performed in duplicate for Cranberry beans. The model comprises a three-step procedure in order to mimic the digestive process in the mouth, stomach (gastric digestion), and small intestine (duodenal digestion. Gastric phase**.** 10.5 mL gastric aliquots prepared by 1.5 mL pepsin solution (10 mg pepsin dissolved into 10 mL 0.1 N HCl) mixed with 10 mL distilled water was added. The samples in beakers were acidified to pH 1.7 with 5 N HCl and incubated at 37°C in a shaking water bath (Memmert, Schwabach, Germany) at 100 rpm for 2 hours. After gastric digestion, aliquots of the post-gastric (PG) digestion were collected from each sample. Intestinal phase. The pH was increased with the addition of 4.5 mL of pancreatin and bile salt mixture (18 mg pancreatin and 112.5 g bile salt dissolved into 4.5 mL distilled water) followed by the addition of dialysis bags filled with 25 mL (the amount required to neutralize the samples at pH 6.5) sodium bicarbonate (21 g NaHCO3 dissolved in 500 mL distilled water). Samples were incubated again in a shaking water bath (100 rpm) at 37°C for another 2 hours to complete the intestinal phase of the *in vitro* digestion process. After the intestinal phase, the solutions in the dialysis bags were taken as the IN sample representing the material that entered the serum, and the solution outside the dialysis bags were taken as the OUT sample representing material that remained in the gastrointestinal tract. Blank was also prepared with identical chemicals but without sample, and underwent the same conditions. The resultant samples are collected in eppendorf tubes and centrifuged at 4°C, 18000 rpm for 10 minutes before store at -20 °C until use. Later, the collected samples (IN and OUT) were assayed for total phenolic content, antioxidant capacity and individual phenolic acids.

2.6 HPLC Analysis of Major Individual Phenolic Compounds

The HPLC analysis was performed using a Shimadzu 20A series ultra-fast liquid chromatograph, coupled with degasser, autosampler, column oven and SPD M20A model PDA detector (UFLC, Shimadzu Corporation, Kyoto, Japan). The chromatographic separations were performed on an Inertsil C18 column (150 mm x 4,6 mm, 3 μm).

A gradient of mobile phase A (water with 0.75 % formic acid) and mobile phase B (methanol with 0.75 % formic acid) was used. The flow rate was 1 mL/ml and the injection volume was 10 μL for each standard mixture and the column temperature was set to 40 °C. A 55 minutes' gradient program was used with the gradient profile as follows: 0–3 min: 5% B, 3–18 min: 40% B, 18–45 min: 80% B, 48–50 min: 100% B, 52-55 min: 5% B.

Identification and quantification of polyphenols were done by comparing uv spectrum and area of the peaks in the extracts with that of 35 standard compounds (gallic acid, 3,4dihydroxybenzoic acid, catechin, 4p-hydroxybenzoic acid, caffeinne, chlorogenic acid, vanilic acid, epigallocatechingallate, caffeic acid, epicatechin, syringic acid, vanilin, p-coumaric acid, ferulic acid, sinapic acid, ethyl 3,4dihydroxybenzoate, rosemarinic acid, resveratrol, rutin, phloridzin, myricetin, trans-cinnamic acid, naringenin, pinobanksin, quercetin, hesperetin, luteolin, kaempferol, apigenin, isorhamnetin, rhamnetin, ladanein, chrysin, pectolinaringenin, pinocembrin and pinostrobin).

2.7 Statistical Data Analysis

All measurements were reported means and standard deviations. For multiple comparisons, data were subject to the statistical analysis using Minitab software Versions 18, Minitab Pennsylvania, USA) for the analysis of variance (ANOVA) and Tukey tests.

3. RESULTS AND DISCUSSION

3.1 Protein Content of Protein Isolates

In this study, protein content of CbPI determined 86.11±2.38% with dry basis. (Deshpande and Cheryan 1984) found the protein content of cranberry beans protein isolates76.2%, (Rui et al. 2011) studied on *Phaeolus Vulgaris.* L variabilities and obtained their protein isolates by isoelectric precipitation, reported protein content of them 89.25%.

3.2 Functional Properties

Dry bean flours can be used as functional ingredients to enhance the nutritional quality of a different kind of processed food products. The application of diverse technological processes to legumes can enhance them apply as an ingredient in fabricated foods. Processing improves the nutritional quality of dry beans by lowering the content of anti-nutritional factors and, at the same time, diversifies their use as ingredients by changing their functional properties (Siddiq et al. 2010).

3.3 Water holding capacity and Oil holding capacity

According to results of present study water holding capacity of freeze dried Cb1PI type was 2.38±0.16 and Cb2PI was 2.34±0.20 g of water/g of protein.

(Siddiq et al. 2010) reported WHC of cranberry bean 2.41±0.21 g water/g protein, which is very close to the value of this study. (Sathe 2002) studied the functional properties of common bean and found the WHC of cranberry 1.73 g water/ g protein.

Water holding capacity is significant for certain product characteristics, such as the moistness of the product, starch retro-gradation, and product staling (Siddiq et al. 2010).

Water holding capacity of bean proteins rely on the type and amount of the protein also as the presence of non-protein components in the preparation. Among the non-proteins, carbohydrates in particular increase WHC of bean protein preparations. In

human and animal nutrition, the dry bean proteins that are of major significance include the water-soluble (albumins) and dilute salt soluble (globulin) storage proteins and some of the metabolic proteins such as the enzyme inhibitors and lectins. In the literature the amount of albumin: globulin for cranberry was 0.52 and it can reduce WHC of cranberry bean, for instance albumin/globulin in faba beans are 0.85 and it means water soluble proteins in small white bean (WHC 4.36 g/g) is higher than cranberry beans, and it may reduce storage time (Sathe 2002).

In this study OHC of Cb1PI and Cb2PI was detected to be 2.24 g oil/g protein and to be 2.42 g oil/g protein respectively. Wile (Sathe 2002) found OHC of cranberry was 1.45g/g and (Siddiq et al. 2010) reported as 1.48 g/g . Higher oil holding capacity is effected by the amount of albumin/globulin, globulin level is low and the level of globulin is high and it may increase the OHC. The differences between functional properties of cranberry bean in literature may be is due to the difference between geographical differences like soil compounds the harvest and cultivation condition.

3.4 Foaming and Emulsifying Properties of CbPI

FC and FS results for both type of CbPI was detected to be 41.2 ml/100ml and to be 40.4% respectively. The values are similar to (Siddiq et al. 2010) which were found the FC of cranberry was 49.6±1.8 and FS was 54.9±2.1. Foaming capacity and stability results of cranberry beans in this study are high in compare with other beans because the protein level is high (Siddiq et al. 2010).

Okaka and Potter (1979) suggested that the superiority of soy flour to cowpea powder in foaming property was due to the high protein content of soy flour. Results reported in these studies demonstrated that not only the amount of native protein in the product but probably also the nature of the protein involved influences the foaming stability of the flour (Siddiq et al. 2010).

The emulsifying activity for of Cb1PI and Cb2PI were 55.08 was 0.47 respectively, and the stability for both was 64%. (Siddiq et al. 2010) reported the EA of cranberry bean 53.4±2.1% and the stability 52.4±2.0%. ES of cranberry beans in this study is high.

Emulsion stability of beans may effect with the amount of fat in beans, if the amount of fat were high the emulsion stability will increase too. The amount of fat in cranberry in the literature was 3.46 g/100g and and higher than other common beans.

3.4.1 Spectrophotometric Analysis

1.1.3 Total phenolic content(TPC) of *Phaseolus vulgaris* types

Total soluble phenolic content (TPC) was determined using the Folin–Ciocalteu reagent and the results are expressed as equivalents of Gallic acid, which is the most commonly used standard in phenolic estimations since Gallic acid found to be more stable and pharmacologically active antioxidant. Besides, it has also been shown quantitatively to be equivalent to most other phenolic and give consistent and reproducible results (Sreeramulu and Raghunath 2010).

The total phenolic contents of cranberry bean varieties and their PI are shown in Table 3.1. All data are given in terms of mg GAE/100g on dry matter basis. The TPC values of Cb1 was 179.94 and Cb2 was 229.33 mg GAE/100g DW in cranberry bean flour while TPC of Cb1PI 114.71 and Cb2PI was 131.69 mg GAE/100g DW. The results show that, TPC of CbPI was lower than the TPC of Cb flour, since in the isolation of proteins of Cb some of the phenolic content might be washed away. TPC of Cb2 is higher than Cb1, and it might be related to its darker color in compare to type 1, this difference is also shown in PIs.

The phenolic compounds contents may vary depending on numerous factors such as varieties, types, growth conditions, metabolic states, initial compound level and intensity of stress (Hengel and Shibamoto 2013). It is difficult to compare the results with those reported in the literature because they express the results in different units, and not always all information is available to recalcute the reported values in mg GAE/g DW, and there are not more studies about cranberry bean phenolic acid content. (Padhi et al. 2017)reported that the TPC of cranberry bean 4.26 ± 0.45 (mg GAE/g DW), their result is higher than current study. (Chen, Dupuis, et al. 2015) Found the TPC content of cranberry bean 2.00–2.06 (mg GAE/g), this results are similar to this study. (Orak et al. 2016) Studied about white types of common bean and the TPC of them ranged between 0.33 and 0.63 mg GAE/g. The color of beans is significant in phenolic compounds level.

Cranberry beans TPC levels are in medium range among other legumes. The darker color of Cb1 may cause its higher phenolic content.

There are significant differences between two types of cranberry beans flour and PIs in statistical analysis.

3.4.2 Total flavonoid content of cranberry beans

The total flavonoid contents of cranberry bean varieties and their PI are shown in Table 3.2. Total flavonoid content of cranberry beans analysis was detected as mg catechin/100gr dry sample. Calibration curve which prepared for measuring TFC of beans is shown in figure 3.3. The total flavonoid content of cranberry beans ranged from 178.41 to 199.43 mg CE/100gr in flour and 82.95 to 87.50 in PIs.(Chen, Dupuis, et al. 2015) reported that TFC of cranberry bean 1.2 (mg CE/g bean). (Singh et al. 2017) found the TFC of cranberry beans between 0.92-4.24 mg CE/g. TFC of cranberry type 1 is higher than type 2, it may be relate to the higher color pigment of type 2, this is also observed in PIs of them. PIs TPC is lower than flour, some of them might be washed away trough protein isolation procedure.

Different factors can effect flavonoid content of legumes for instance, the method of extraction, growth condition of plants, stress level, soil condition and etc. In the case of statistical analysis there are significant difference between the TFC of two types of cranberry beans and their protein isolates.

Table 3.1 : Average phenolic and flavonoid contents on dry basis. Means of triplicated analyses are given with two significant digits and ± standard deviations.

	Cb1	Cb2	CbPI1	CbPI2
TFC	171.88 ± 0.4^{a}	198.58 ± 1.2^{b}	75 ± 0.8^{c}	88.07 ± 0.8^{d}
TPC	179.94 ± 1.09^{a}	229.33 ± 0.21^{b}	114.71 ± 0.36^{c}	131.69 ± 0.65^{d}

TPC (Total phenolic content) of cranberry beans flour and protein isolates (mg GAE/100g)

TFC (Total flavonoid content) of cranberry beans analysis gives in mg Catechin/100gr DW, Cb1: Cranberry type 1 (oval), Cb2: Cranberry type 2(kiraz), CbPI1: Cranberry protein isolates type 1(oval), CbPI2: Cranberry protein isolates type 2(kiraz)

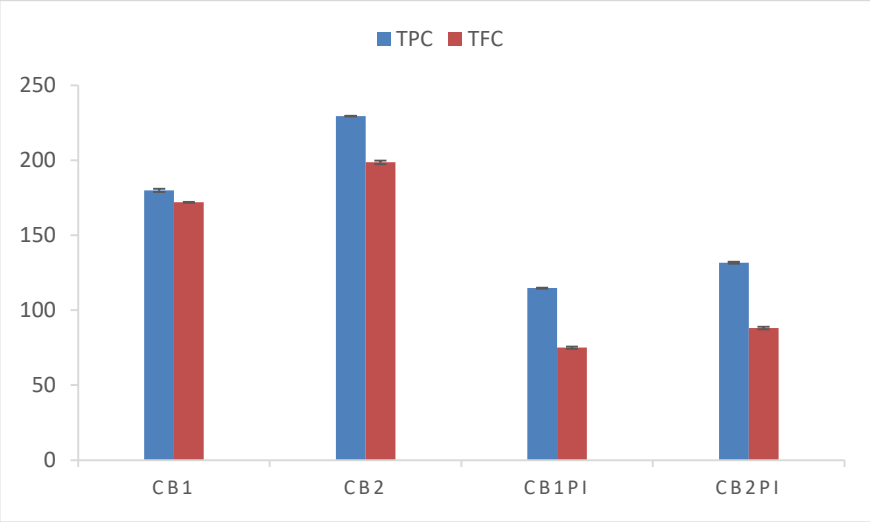


Figure 3.1 : Average phenolic and flavonoid contents on dry basis. Means of triplicated analyses are given with two significant digits and ± standard deviations.

TPC (Total phenolic content) of cranberry beans flour and protein isolates (mg GAE/100g)

TFC (Total flavonoid content) of cranberry beans analysis gives in mg Catechin/100gr DW, Cb1: Cranberry type 1 (oval), Cb2: Cranberry type 2(kiraz), CbPI1: Cranberry protein isolates type 1(oval), CbPI2: Cranberry protein isolates type 2(kiraz)

3.5 Antioxidant Activity

An antioxidant can be defined as substance that opposes oxidation and protecting cells from the damage. Dietary antioxidants, including polyphenolic compounds, are believed to be the effective nutrients in the prevention of these oxidative stress related diseases such as atherosclerosis, diabetes mellitus, chronic inflammation, neurodegenerative disorders, and certain types of cancer (Huang, Ou, and Prior 2005). Since there are many assays which differ from each other in terms of reaction mechanisms, oxidant and target/probe species, reaction conditions, and expression of results have been developed and tested in the literature, it is important to quantify the total antioxidant capacity using more than one method in order to reach reliable results (Karadag, Ozcelik, and Saner 2009b).

The antioxidant capacity of samples was determined using two different assays, namely DPPH and CUPRAC. The method of scavenging the stable DPPH radical is widely used to evaluate antioxidant activities in a relatively short time compared with other methods (Duh, Tu, and Yen 1999). The effect of antioxidants on DPPH radical scavenging was thought to arise from their hydrogen donating ability. DPPH radical is a stable free radical and accepts an electron or hydrogen radical to become a stable molecule. The reduction capability of DPPH radicals was determined by the decrease

in its absorbance at 517 nm induced by antioxidants. It is also visually noticeable as a discoloration from purple to yellow. Cupric ion reducing antioxidant capacity (CUPRAC) assay is also a spectrophotometric method, which can simultaneously measure hydrophilic and lipophilic antioxidants at physiological pH as the reagent used, neocupreine, is soluble both in water and organic media (Apak et al. 2005).

The results of this study showed TAC of Cranberry beans flour type 1 in average measure is 191.90 and type 2 is 114.14 and the protein isolates of them is 86.67 in type 1 and 73.99 in type 2. An obvious conclusion can be derived from these results is that the protein isolates have lower AA in compare to the AA of cranberry beans flour. Studies about the antioxidant activity of cranberry beans is limited.(Chen, Dupuis, et al. 2015) found the AA of cranberry beans between 0.80–10.7μmol TE/g DW by DPPH test. The antioxidant activity results by CUPRAC test are 165.6 to 223.46 in Cb flour and in protein isolates are between 85.85-115.21 expressed as mg Trolox equivalents/100g sample. There are not any studies over CUPRAC test on Cranberry beans until now.

Table 3.2 : Antioxidant capacity of Cb1, Cb1, CbPI1 and CbpI2 by two different methods(DPPH and CUPRAC), datas expressed as mg Trolox equivalents/100g DW

	Cb1	Cb2	CbPI1	CbPI2
DPPH	191.09 ± 1.09^{a}	114.14 ± 0.11^{b}	86.67 ± 0.35^{c}	73.99 ± 1.01^{d}
CUPRAC	223.46 ± 0.73^{a}	165.6 ± 0.49^{b}	115.21 ± 0.49^{c}	85.85 ± 0.24^{d}

CUPRAC: Cupric reducing antioxidant capacity, DPPH: 2,2-diphenyl-1-picrylhydrazyl, Cb1: Cranberry bean type 1 (oval), Cb2: Cranberry type 2(kiraz), CbPI1: Cranberry protein isolates type 1(oval), CbPI2: Cranberry protein isolates type 2(kiraz)

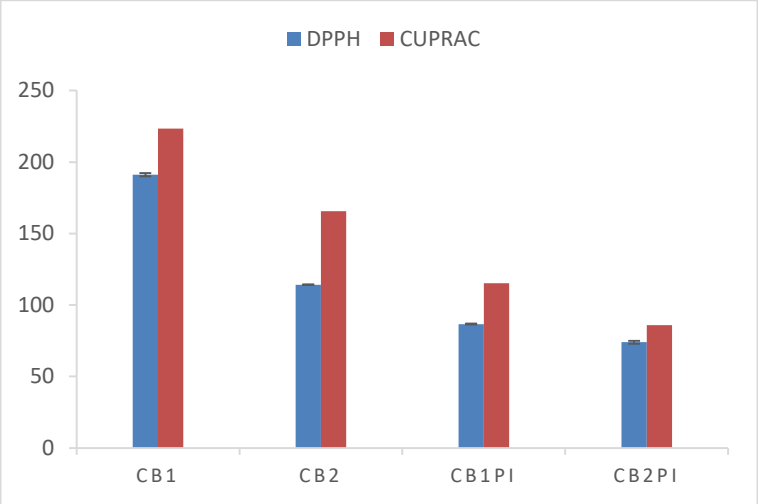


Figure 3.2 : Antioxidant capacity of Cb1, Cb1, CbPI1 and CbpI2 by two different methods(DPPH and CUPRAC), datas expressed as mg Trolox equivalents/100g DW

3.6 *In vitro* Gastrointestinal (GI) Digestion

In recent years, bioaccessibility studies have given evidence of the absorption of any phytochemicals and have shown that uptake varies widely, depending on the type of compound and/or food matrix (Manach et al. 2005). In vivo studies on humans and animals are not preferred due to the limitations of complexity, expensiveness and ethical reasons. On the other hand, *in vitro* digestion method is a rapid and simple to evaluate phytochemical stability in proceed food (Akillioglu, Karakaya, and Biotechnology 2010). With the scope of this study, the bio accessibility of this local cranberry of Turkey is described for the first time.

3.6.1 Total Phenolics after GI Digestion

Results for total phenolic content analysis after *in vitro* digestion were expressed as mg gallic acid equivalents per 100g standard for each GI digestion samples obtained from two fractions (IN and OUT) are shown in table 3.4. The standard calibration curve of gallic acid shown in Figure 3.4 was prepared in concentrations between 0.01-0.2 mg/ml and obtained equation from the calibration curve was used to calculate the gallic acid equivalent values of the samples measured spectrophotometrically.

The analysis of total phenolic content of digested samples showed 3-fold increase comparison with the raw material (INITIAL). TPC of Cb2 is higher than Cb1 after digestion and in IN fraction and OUT fraction of them is same as previous ones.

The basic reason of the increase in OUT fractions may be that bound phenolic compounds may become soluble in dialyze fluids especially due to break down reactions during the simulated gastric and duodenal phase. Although the released phenolic compounds may have interacted with peptides resulting from protein digestion, they exhibited higher radical scavenging properties than when bound to unhydrolyzed, denatured protein in the undigested cranberry beans (Apea-Bag *et al*., 2016).

During gastro-intestinal digestion, polyphenols may either interact with other food constituents such as sugars, lipids and fibre, that causes reduction or improvement in their bioaccessibility (Palafox-Carlos, Ayala-Zavala, & Ganzalez-Aguilar, 2011; Toydemir et al., 2013)

The theory behind the Folin-Ciocalteau method, it strongly relies on the reduction of the Folin-Ciocalteau reagent. For this reason, any reducing component such as small peptides or reducing sugars formed by digestion or fermentation can interfere in the Folin-Ciocalteau assay (Ikawa *et al*., 2003). However, in this study there was no big differences in the observed TPC values between Initial and OUT fractions of all samples.

Table 3.3: TPC of beans after *in vitro* digestion

	INITIAL	IN	OUT
Cb1	179.94 ± 1.09^{a}	156.39 ± 1.51^{b}	490.80 ± 1.27^{a}
Cb2	229.33 ± 0.21^{b}	220.19 ± 2.64^{c}	488.41 ± 7.66^{a}

The terms represent; INITIAL: TPC of the sample before digestion; IN: TPC of the sample after digestion in small intestines. OUT: TPC of the sample that cannot be recovered by GI tract. All values are given as mean values of triplicate runs with its standard deviations. All values are expressed as mg Gallic acid equivalents per 100g dry weight.

Percent recovery of total phenolic contents obtained by Folin-Ciocalteu assay with respect to the GI digestion fractions (IN, OUT) by assuming the initial values as 100%. The results are summarized in Table 3.5 and shown in figure 3.5.

Table 3.4: Total phenolic content recoveries % for IN and fractions.

	INITIAL	IN	OUT
Cb1	100	86.9%	214.0%
Cb2	100	122.4%	213.0%

The terms represent; INITIAL: TPC of the sample before digestion; IN: TPC of the sample after digestion in small intestines. OUT: TPC of the sample that cannot be recovered by GI tract. All values are given as mean values of triplicate runs.

Recovery of phenolic compounds are quite high. When recovery ratios of OUT fractions were compared, the Cb1 (86.9%) recovery is higher than Cb2 (122.4%).

3.6.2 Total Flavonoid Content (TFC) after digestion

Total flavonoid content of initial samples, IN (dialyzed) and OUT (non-dialyzed) fractions and their comparison with TFC of extracts are given in Table 3.6. The standard calibration curve of Catechin shown in Figure 3.4 was prepared in concentrations between 0.01-0.2 mg/ml and obtained equation from the calibration curve was used to calculate the Catechin equivalent values of the samples measured spectrophotometrically.

The analysis of total flavonoid content of digested samples showed low decrease amount of TFC in comparison with the raw material (INITIAL). TFC of Cb1 is higher Cb2 after digestion and, this row repeated in IN and OUT fraction of them.

Table 3.5: TFC Cranberry bean flour after *in vitro* digested, expressed as CAE per 100g dw

	INITIAL	IN	OUT
Cb1	171.88 ± 0.4^{a}	23.82 ± 1.5^{c}	67.65 ± 0.6^{a}
Cb2	198.58 ± 1.2^{b}	18.03 ± 2.2^{c}	51.98 ± 3.1^{b}

The terms represent; Initial, as initially determined from cowpea; IN, dialyzed free fraction after intestinal digestion; OUT, non-dialyzed free fraction after intestinal digestion. Data represents average values present error indicators as standard deviation of 2 independent samples.

TFC recovery of Cb1 is 13.9% and Cb2 is 9.1%, in compare with the recovery of TPC of beans TFC recovery is quite low.

Table 3.5: Total flavonoid content recoveries % for IN and fractions.

	INITIAL	IN	OUT
Cb1	100	13.9	39.4
Cb2	100	9.1	26.2

INITIAL: TPC of the sample before digestion; PG: TPC of the sample after digestion in stomach; IN: TPC of the sample after digestion in small intestines. OUT: TPC of the sample that cannot be recovered by GI tract. All values are given as mean values of triplicate runs.

(Kumaran and Joel karunakaran 2006), phenolic and flavonoid compounds; altered chemical structure, increased or decreased solubility, or interaction with other compounds may occur during gastrointestinal digestion and may affect bioavailability. (Tenore et al. 2013) suggested that some flavonoid glycosides have good stability against gastric acidic environment, but have great instability under alkaline intestinal digestion conditions. Chen et al. (2016) reported that the imbalance of these compounds under alkaline conditions can be attributed to the fact that they undergo many changes such as oxidation, polymerization and transformation. In addition, Saura- Calixto et al. (2007) reported that the reduction in the concentration of phenolic compounds under basic conditions is related to the complex formation of these compounds between metal ions, proteins and / or fibers.

3.6.3 Total Antioxidant Capacities After GI Digestion

The antioxidant capacity was determined by DPPH and CUPRAC methods in Cranberrry beans flour, samples collected from fractions (IN and OUT) after *in vitro* GI digestion. Standard calibration curve was prepared by using Trolox and it is given in Figure 3.5 and figure. The results were expressed as mg TEAC/100g standard. The standard calibration curve was prepared between 0.01- 0.4 mg/ml concentrations and the equation obtained from the curve was used to calculate the antioxidant activity of the sample measured spectrophotometrically. The results are shown in Table 3.8.

The anaylsis of total antioxidant capacities of digested samples showed high decrease in DPPH test, on the other hand TAC of beans increased in OUT fraction in CUPRAC test in comparison with the raw material (INITIAL). Overall the TAC of samples in OUT fraction is higher than IN fraction. The results of CUPRAC test in compare to initial sample is quite high in out fraction.

The basic reason of the increase in OUT fractions may be that bound phenolic compounds may become soluble in dialyze. This also may be attributed to the release of extractable phenolic compounds bound to proteins during the simulated gastric and duodenal phase. Although the released phenolic compounds may have interacted with peptides resulting from protein digestion, they exhibited higher radical scavenging properties than when bound to non-hydrolyzed, denatured protein in the undigested cranberry bean (Apea-Bag *et al*., 2016).

Table 3.6: Antioxidant activity of cranberry beans by DPPH method after *in vitro* digestion.

DPPH	INITIAL	IN	OUT
Cb1	191.09 ± 1.09^{a}	3.31 ± 0.99^{c}	3.40 ± 1.99^{a}
Cb2	114.14 ± 0.11^{b}	1.73 ± 0.46^{c}	33.80 ± 0.22^{b}

Table 3.7: Antioxidant activity of cranberry beans by CUPRAC method after *in vitro* digestion.

CUPRAC	INITIAL	IN	OUT
Cb1	223.46 ± 0.73^{a}	148.49 ± 4.85^{d}	722.49 ± 1.60^{a}
Cb2	165.6 ± 0.49^{b}	222.91 ± 0.57^{d}	456.39 ± 6.71^{b}

INITIAL: 2,2-diphenyl-1-picrylhydrazyl radical scavenging antioxidant capacity of the sample before digestion; IN: 2,2-diphenyl-1-picrylhydrazyl radical scavenging antioxidant capacity of the sample after digestion in small intestines. OUT: 2,2-diphenyl-1-picrylhydrazyl radical scavenging antioxidant capacity of the sample that cannot be recovered by GI tract. All values are given as mean values of triplicate runs with its standard deviations. All values are expressed as mg Trolox equivalents per g dry weight.

Recovery percent of antioxidant capacity after GI digestion are shown in figure 3.8. Recovery of phenolic compounds are quite low in DPPH test results, the recovery of antioxidants of Cb1 is 2.32% and Cb2 is 1.21%. The lower recovery of Cb1 in compare to Cb2 may be due to its lower antioxidant capacity, the results of TAC recovery of CUPRAC test is higher than DPPH test results, the recovery of TAC of Cb1 is 66% and Cb2 is 134%.

Table 3.8: antioxidant recoveries after GI degestion detected of antioxidant detected by DPPH and CUPRAC test

DPPH	INITIAL	IN	OUT
Cb1	100	2.32	3.02
Cb2	100	1.21	30.04

CUPRAC	INITIAL	IN	OUT
Cb1	100	66.4	323.3
Cb2	100	134.6	275.6

Antioxidant activity recovery of cranberry beans by CUPRAC and DPPH method after *in-vitro* digestion.

INITIAL: 2,2-diphenyl-1-picrylhydrazyl radical scavenging antioxidant capacity of the sample before digestion; IN: 2,2-diphenyl-1-picrylhydrazyl radical scavenging antioxidant capacity of the sample after digestion in small intestines. OUT: 2,2-diphenyl-1-picrylhydrazyl radical scavenging antioxidant capacity of the sample that cannot be recovered by GI tract. All values are given as mean values of triplicate runs with its standard deviations. All values are expressed as mg Trolox equivalents per g dry weight.

3.7 Phenolic Profile of Cranberry Bean Flour Extracts by HPLC after GI *in Vitro* Digestion

Identification and quantification of polyphenols were done by comparing uv spectrum and area of the peaks in the extracts with that of 35 standard compounds (gallic acid, 3,4dihydroxybenzoic acid, catechin, 4p-hydroxybenzoic acid, caffeinne, chlorogenic acid, vanilic acid, epigallocatechingallate, caffeic acid, epicatechin, syringic acid, vanilin, p-coumaric acid, ferulic acid, sinapic acid, ethyl 3,4dihydroxybenzoate, rosemarinic acid, resveratrol, rutin, phloridzin, myricetin, trans-cinnamic acid, naringenin, pinobanksin, quercetin, hesperetin, luteolin, kaempferol, apigenin, isorhamnetin, rhamnetin, ladanein, chrysin, pectolinaringenin, pinocembrin and pinostrobin).

Nine major phenolics were determined by HPLC system: Gallic Acid, ECGC, 4-hydroxybenzoic acid, syringic acid, caffeinne, catechin, *t*-cinnamic acid, chlorogenic acid, ferulic Acid. They are shown in table 3.8 and table 3.9. the changes in peaks are so close in both type of beans.

ECGC ,4-hydroxybenzoic acid, syringic acid, caffeinne, was not detected in initial samples but detected after digestion in IN and OUT fractions and Gallic Acid and t-cinnamic Acid was in very low amount in initial sample but increased in IN and OUT fraction remarkably. ECGC is the major phenolic with 67.34 ppm level in IN fraction. The amount of phenolics increased after GI digestion, PH and enzymes effect may increase phenolic compounds during GI digestion. chlorogenic Acid amount decreased during GI digestion.

The results of HPLC detection of phenolic compounds in that study is similar to two other study (Fan and Beta 2017).

Table 3.9 : Phenolic profile of Cb1 and Cb2 determined by HPLC

	Cb1		Concentration (ppm)		
Peak No	Retention Time	Compound Name	INITIAL	IN	OUT
1	2.15	Gallic Acid	**< 0.1**	**23.85**	**61.58**
2	2.43	ECGC	**N.D.**	**67.34**	**80.09**
3	4.18	4-hydroxybenzoic Acid	**N.D.**	**15.99**	**22.72**
4	5.07	Syringic Acid	**N.D.**	**9.18**	**10.12**
5	5.45	Caffeinne	**N.D.**	**4.54**	**57.85**
6	8.21	Catechin	**74.62**	**17.57**	**54.79**
7	10.50	t-cinnamic Acid	**< 0.1**	**17.40**	**28.33**
8	11.79	Chlorogenic Acid	**52.45**	**19.54**	**35.97**
9	13.71	Ferulic Acid	**2.37**	**9.39**	**18.80**

	Cb2		Concentration (ppm)		
Peak No	Retention Time	Compound Name	INITIAL	IN	OUT
1	2.15	Gallic Acid	**< 0.1**	**35.67**	**71.77**
2	2.43	ECGC	**N.D.**	**30.86**	**62.72**
3	4.18	4-hydroxybenzoic Acid	**N.D.**	**7.78**	**13.77**
4	5.07	Syringic Acid	**N.D.**	**6.93**	**11.75**
5	5.45	Caffeinne	**N.D.**	**15.27**	**66.41**
6	8.21	Catechin	**52.21**	**15.52**	**15.31**
7	10.50	t-cinnamic Acid	**< 0.1**	**15.29**	**18.58**
8	11.79	Chlorogenic Acid	**56.35**	**23.32**	**40.33**
9	13.71	Ferulic Acid	**10.79**	**11.63**	**19.75**

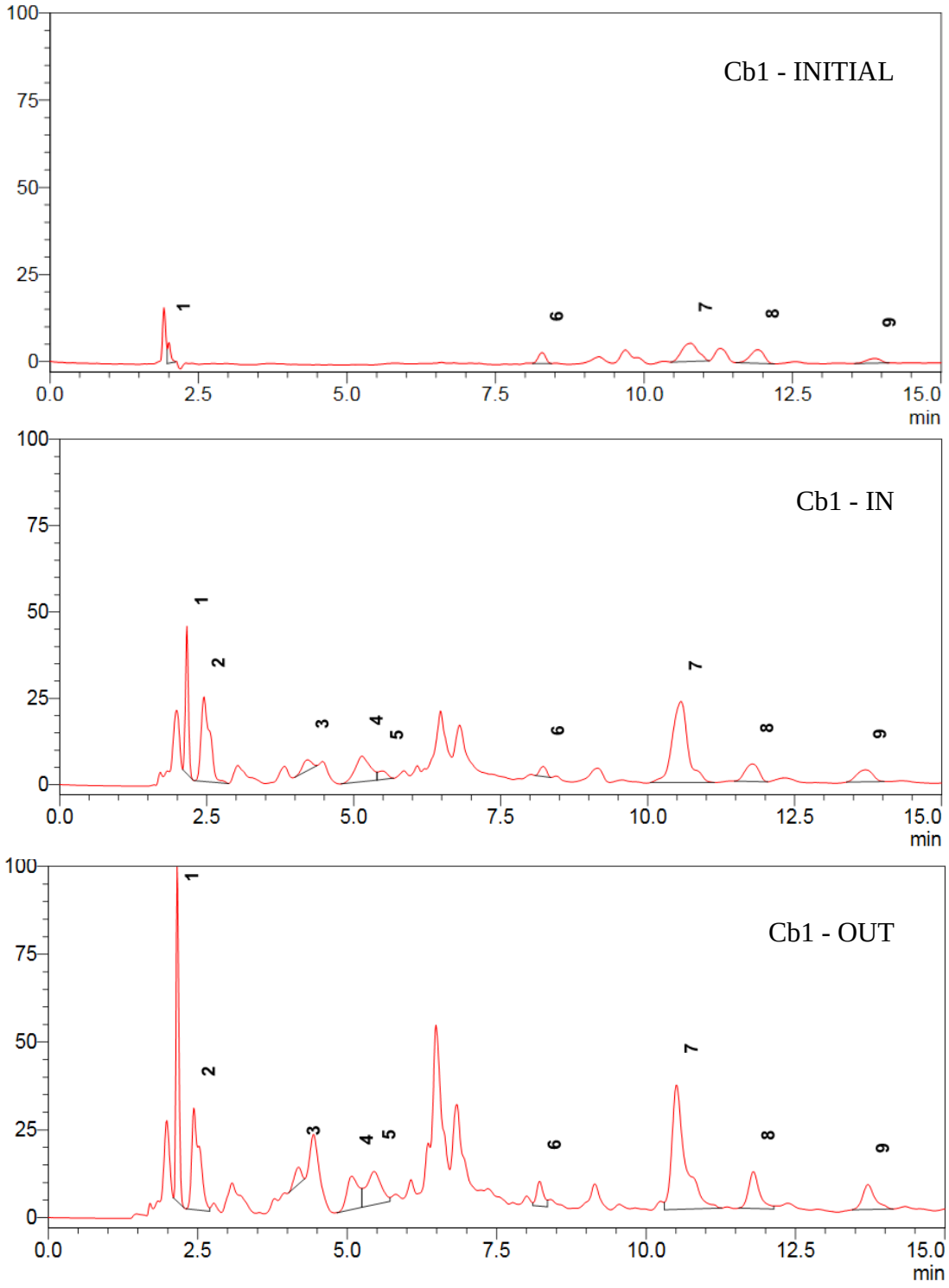


Figure 3.3: chromatogram peaks of phenolic acids of Cb1 by HPLC

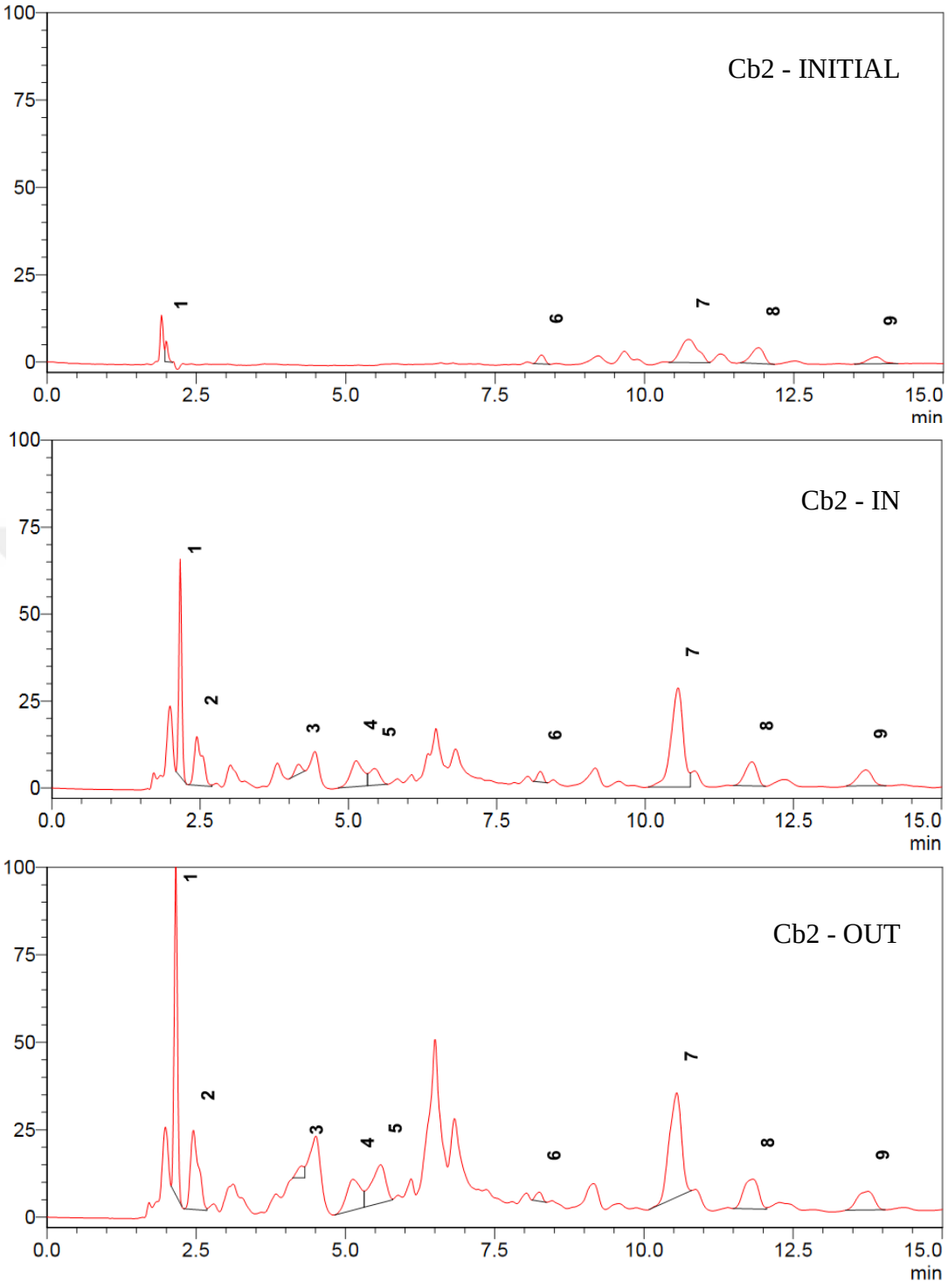


Figure 3.4: chromatogram peaks of phenolic acids of Cb1 by HPLC

CONCLUSIONS AND RECOMMENDATIONS

In this study, functional properties of proteins, TPC, antioxidant capacity, bio-accessibility of phenolic acids, phenolic profile of cranberry bean flour and protein isolates were determined and compared after GI digestion using *in vitro* models.

Legumes are considerable interest of academia due to sufficent nutritional composition, and alternative to animal originated foods. On the other hand, altering functional properties of proteins are gaining importance with noval methods and sustainable ways, *Phaseolus Vulgaris* L. (Cranberry Bean) has high protein content with like as other legumes. Although bean varieties are widely distributed around the world, data on the functional properties, bioactive compounds, bioaccessibility of polyphenols and phenolic profile of them are limited. The objective of this study is to study the functional properties of cranberry beans, analyze bioactive compounds value and, bioaccessibility, Giving the bioactive compounds profile of cranberry beans to increase interest in their use for food formulations.

Proteins were isolated by isoelectric method, Total phenolic content was determined by Folin-Ciocalteu method and total antioxidant capacity by DPPH and CUPRAC assays were performed before and after digestion and phenolic profile determined by HPLC. The experiments were carried out to two different types of cranberry beans which were harvested from Nigde, Turkey.

Functional parameters are very important in food industry, plant based protein are started to replace animal proteins and searching functional properties of plant proteins are important. WHC of CbPI is 2.36 g of water/g of protein. In this study OHC is detected 2.33 g of oil g of protein isolate DW. Low WHC of proteins in cranberry beans may have benefial effect on storage condition, and higher OHC of this special local cranberry bean in compare to other cranberries from different region have effect on rancidity of product. The emulsifying activity in both type of beans was 50.95 and stability was 64%, by that EC and ES this type of cranberry may have good effect on bakery products and mayonnaise. Foaming capacity of CbPI was 40.4 to 41.2% and

emulsifying capacity was 0.47 to 55.8%. In both types of beans, the stability of emulsifying capacity was 64%. EC and ES of cranberry is high in compare to other beans.

This study supports that, protein isolate extracted isoelectric precipitation from Cb which have possible application as functional ingredient during food manufacturing. Especially, foaming and emulsifying activities and stabilities are remarkable functional properties comparing to OHC and WHC. WHC is functional property of proteins, which are important for soups, sauces, bakery products. OHC plays very important role like as mayonnaise or similar products due to emulsifying activity has close correlation with oil absorption capacity. CbPI could be promising emulsifying agent food, which needs high emulsification, and emulsion stability such as meat analogs, soups, salad dressings, mayonnaise, and cakes, plant based beverages. Experimental studies support that, CbPI might be great potential to find application in food products such as marshmallows, coffee whiteners, cakes, whipped toppings due to impressive foaming properties. Beside functional properties phenolic compounds, antioxidant capacity, and phenolic profile of beans are important. Nowadays, these properties of foods can attract people attention to plant protein consumption.

However, the quantity of these particular phenolic compounds can alter relying on the type of the legumes. The most frequently phenolic acid in legumes is p-Hydroxybenzoic acid and after that coumaric, procatechuic and ferulic acid. In pea, and chickpea the major phenolic acid is gallic acid (147-138 mg/100g FW, respectively) the major phenolic acid in lentil is coumaric acid (321-343 mg/100g DW) ben bean is trans-ferulic acid (341-367 mg/100g DW). protocatechuic acid (31 mg/100 g FW) in pea.

With the scope of this study, the bio accessibility of this local cranberry bean of Turkey is described for the first time. The bioaccessability of phenolic compounds (investigated by folin- Ciocalteu method) and antioxidants capacity (investigated by CUPRAC method) increased remarkably after GI digestion.

Nine major phenolics were determined by HPLC system: Gallic Acid, ECGC, 4-hydroxybenzoic Acid, Syringic Acid, Caffeinne, Catechin, t-cinnamic Acid, Chlorogenic Acid, Ferulic Acid. They are shown in table 3.8 and table 3.9. the changes in peaks are so close in both type of beans.

ECGC ,4-hydroxybenzoic Acid, Syringic Acid, Caffeinne, was not detected in initial samples but detected after digestion in IN and OUT fractions and Gallic Acid and t-cinnamic Acid was in very low amount in initial sample but increased in IN and OUT fraction remarkably. ECGC is the major phenolic with 67.34 ppm level in IN fraction. The amount of phenolics increased after GI digestion, PH and enzymes effect may increase phenolic compounds during GI digestion. Chlorogenic Acid amount decreased during GI digestion.

In conclusion, this study focused on the measuring phenolic compounds, antioxidant activity, bioaccessibility and antinutritional compounds of cranberry bean. Although the results obtained with the model of simulated *in vitro* GI digestion cannot directly predict the human *in vivo* conditions, still this model is helpful for investigating the bioaccessibility of phenolic compounds.

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